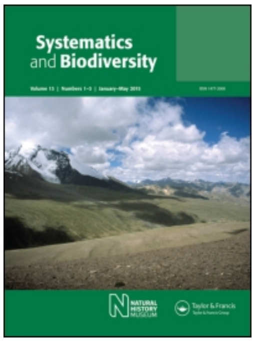


Systematics and Biodiversity

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tsab20

Systematic analysis of the genus *Eriocaulon* L. in India based on molecular and morphological evidence

Ashwini M. Darshetkar, Mandar N. Datar, K.M. Prabhukumar, Soo-Yong Kim, Shubhada Tamhankar & Ritesh Kumar Choudhary

To cite this article: Ashwini M. Darshetkar, Mandar N. Datar, K.M. Prabhukumar, Soo-Yong Kim, Shubhada Tamhankar & Ritesh Kumar Choudhary (2021): Systematic analysis of the genus *Eriocaulon* L. in India based on molecular and morphological evidence, Systematics and Biodiversity, DOI: 10.1080/14772000.2021.1914764

To link to this article: https://doi.org/10.1080/14772000.2021.1914764

Systematics and Biodiversity (2021), **0(0)**: 1–31

Taylor & Francis
Taylor & Francis Group

Check for updates

Research Article

Systematic analysis of the genus *Eriocaulon* L. in India based on molecular and morphological evidence

ASHWINI M. DARSHETKAR[1]*, MANDAR N. DATAR[1], K.M. PRABHUKUMAR[2#], SOO-YONG KIM[3], SHUBHADA TAMHANKAR[1] & RITESH KUMAR CHOUDHARY[1]*

[1]Agharkar Research Institute, G.G. Agarkar Road, Pune, 411 004, Maharashtra, India
[2]Plant Diversity Systematics & Herbarium Division, CSIR-National Botanical Research Institute, 236, Rana Pratap Marg, Lucknow, 226 001, Uttar Pradesh, India
[3]International Biological Material Research Center, Korea Research Institute of Bioscience & Biotechnology, Daejeon, 34141, Republic of Korea

(Received 10 August 2020; accepted 6 April 2021)

The present study is the first-ever comprehensive molecular systematic study of Indian *Eriocaulon* species based on the critical appraisal of the freshly collected 552 accessions of 66 *Eriocaulon* spp. The genus *Eriocaulon* L. (Eriocaulaceae) is one of the most diverse genera of Eriocaulaceae and around one-quarter of the world's representation is in India. The taxonomy of the genus has been challenging due to high intraspecific and low interspecific variation. With about 70% of Indian species endemic to the country, sparse representation in earlier phylogenetic studies did not provide phylogenetic resolution of Indian species. A critical analysis of morphology including microscopic seed surface characters of 55 species is presented here for the first time. Phylogenetic analyses based on ITS and *trnL–F* data yielded three major clades of Indian *Eriocaulon* species. Based on the morphological and molecular data, *Eriocaulon baramaticum*, *E. govindiana*, *E. gulnarparianum*, *E. idukkianum*, *E. maharashtrense* and *E. pradeepii* have been synonymized. Also, *Eriocaulon rhodae* has been reinstated. Molecular data exhibited little congruence with the earlier available taxonomic treatments for Indian species. Several morphological characters were mapped onto a phylogeny and none were found to characterize monophyletic groups.

Key words: India, phylogeny, pipeworts, reinstatement, species complexes, systematics

Introduction

Eriocaulon L. is one of the largest genera of the family Eriocaulaceae, distributed worldwide in tropical and subtropical regions with about 481 species and diversity centres in South America, Africa and the Indian subcontinent (Govaerts, 2020; Judd et al., 1999; Leach, 2000; 2017). The genus is characterized by the presence of diplostemonous flowers, glandular petals, stigmas in a carinal position and a gynoecium without any appendages (Körnicke, 1863; Rosa & Scatena, 2007; Ruhland, 1903). In India, the genus is represented by 105 taxa distributed in two biodiversity hotspots, the Western Ghats and Eastern Himalayas (Ansari & Balakrishnan, 1994; 2009; Chandore et al., 2019; Darshetkar et al., 2017; 2019; Francis et al., 2020; Khanna & Kumar, 2019). The genus shows high diversity and endemism in India, and around 70% species are endemic (Govaerts, 2020). Moreover, the family has the highest threatened species percentage (i.e. 25%) in the Western Ghats flora (Kumar et al., 2011) which can be identified as the centre of diversity for the genus due to its highest species richness.

The pioneering work on Indian *Eriocaulon* species was carried out by Hooker (1893) who recognized 43 species from British India (India with Western Tibet), of which 14 species were newly described. Noting high levels of intraspecific diversity and low interspecific variation within the genus, Hooker referred them as 'the most difficult of classification, presenting no good

Correspondence to: Ritesh Kumar Choudhary. E-mail: rkchoudhary@aripune.org
*Also affiliated to Savitribai Phule Pune University, Pune, 411 007, India.
#Previously with Plant Systematics & Genetic Resources Division, Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Malappuram - 676 503, Kerala, India.

ISSN 1477-2000 print / 1478-0933 online

https://dx.doi.org/10.1080/14772000.2021.1914764

Published online 04 Jun 2021

sectional characters'. Moreover, he found the dimerous and trimerous character of flowers deceptive which might lead to misidentification of the species. Therefore, he grouped Indian *Eriocaulon* species mainly based on their habitats, viz. Aquatics and Terrestrial or Marsh plants. In addition, hairiness of bracts and receptacles were also recognized as distinguishing characters but ultimately, he failed to provide any sectional treatment for the Indian species. Ruhland (1903) recognized 47 species from India and grouped them into sections based on the geographic distribution and number of floral parts. Further, Indian Eriocaulaceae was studied by Fyson (1919–1923) who classified Indian *Eriocaulon* species into eight natural sections based on characters such as hairiness of receptacles, involucral and floral bracts, shape of female sepals, size of male petals and colour of anthers. He also indicated that to classify the species, it is necessary to determine the characters liable to vary with age or environmental conditions. Later, Nair (1987) revealed the significance of seed coat morphology in identification of *Eriocaulon* species. However, he studied only 19 species of the genus. Zhang (1999) gave an account of 74 South East Asian species of the genus and examined the seed appendages of 65 selected species. He highlighted the importance of seed coat surface in the taxonomy of *Eriocaulon* species and also studied the anatomy of peduncles. Based on a cladistic analysis of 54 morphological characters, he recognized two subgenera, viz. *Trimeranthus* Nakai comprising seven sections and 53 species, and *Spathopeplus* Körn. having three sections and 21 species. He also recognized four groups based on female sepals characters. The study gave an account of 21 *Eriocaulon* species which are also reported from India.

A recent revisionary study on Indian species by Ansari and Balakrishnan (1994, 2009) reported 80 species from India, grouped into 12 informal sections. The study was based on herbarium specimens available in major Indian herbaria. The sectional treatment was based on morphological characters, including seed coat characters. Five species were left untreated due to lack of good specimens. They recognized that their sections may not necessarily form phylogenetic groups (*sic*) but can help in identification of the species.

In the last decade, several new Indian *Eriocaulon* species have been described, mostly from the Western Ghats (Anto & Reshma, 2017; Biju et al., 2012; 2018; Chandore et al., 2019; Darshetkar et al., 2017; 2019; Francis et al., 2020; Khanna and Kumar, 2019; Manudev et al., 2017; Nampy et al., 2011; Naveen Kumar et al., 2017; Paithane et al., 2017; Rashmi & Krishnakumar, 2014; Shimpale & Yadav, 2010; Sunil et al., 2013, 2015, 2017; Sunil & Kumar, 2015; Swapna et al., 2012; Vivek et al., 2010) which takes the count of Indian taxa to 105. However, none of the above-mentioned studies gave a full account of Indian *Eriocaulon* species.

Previous molecular phylogenetic studies on Eriocaulaceae suggested *Eriocaulon* to be monophyletic (Andrade et al., 2010; Giulietti et al., 2012; Unwin, 2004) but all these studies were based on sparse sampling of *Eriocaulon* species. Several studies attempted to resolve phylogenetic relationships between members of the subfamily Paepalanthoideae and again considered very few species of *Eriocaulon*, mostly as an outgroup (Andrade et al., 2010; Echternacht et al., 2014; Giulietti et al., 2012; Rosa and Scatena, 2003; 2007; Trovó et al., 2013; Unwin, 2004; Watanabe et al., 2015). No attempts have been made to resolve phylogenetic relationships within this widespread genus except a few sporadic studies such as Davies et al. (2007) who tried to resolve taxonomy of the *E. carsonii* complex using morphometry and AFLP markers. They identified five distinct taxa from Australian mound springs and described two new species and subspecies. Recently, Larridon et al. (2019) carried out the first molecular phylogenetic study for the genus, employing four plastid (viz. *matK*, *rbcL*, *rpoB* and *rpoC1*) and one nuclear marker (PHYC). The study, however, included only four species from India, none of them endemic, and a sparse representation from the other parts of the world. The results of the study exhibited little congruence with the earlier morphology-based classifications proposed by Fyson (1919–1923) and Zhang (1999). The study also suggested origin of the genus to be around 10 mya based on the secondary calibrations available from Poales studies. Despite high endemism and species richness in India, systematics of the genus *Eriocaulon* in India is poorly understood.

This study, therefore, (1) makes an effort to understand the phylogenetic relationships using both molecular and morphological data for the genus *Eriocaulon*, with an emphasis on Indian species, (2) resolves some species complexes, (3) analyses seed surface traits of 55 species, (4) reports rediscovery of a species and reinstates *Eriocaulon rhodae*, and (5) analyses morphological characters which were used in earlier morphology-based classification systems.

Materials and methods

Taxon sampling

Several national (AHMA, ASSAM, BSI, CAL, CALI, GUBH, MH, and SUK), and international (E, HHBG, HZU, K, KRIB, P, W and ZM) herbaria were consulted, either virtually or in person. Voucher specimens were

critically examined to understand the inter- and intraspecific variations, and distribution details of the *Eriocaulon* taxa (Table S1). This was followed by extensive fieldwork in the Western Ghats (Goa, Karnataka, Kerala, Maharashtra and Tamil Nadu states) during the years 2014 – 2018. Field explorations were also carried out in the Eastern Himalayas (Meghalaya) and Orissa to collect the disjunctly located Indian *Eriocaulon* species (Fig. 1). A total of 552 accessions were collected. Attempts were also made to collect the species from their type locations with permission from the biodiversity board (MSBB/desk-5/NOC/85/14-15/798 dated 10/12/2014). Field photographs were taken, and geographic coordinates were recorded for all the collected accessions.

Morphological studies

As stated earlier, the genus is represented by 105 taxa in India (Table S4). They inhabit marshy areas, streams, seasonal pools and occur in rice fields, on cliffs and lateritic plateaus (Fig. 1). Some species exclusively occur at low altitude while some are restricted to high altitudinal areas. The collected specimens were deposited in the Herbarium of Agharkar Research Institute, Pune (AHMA). Accession numbers of voucher specimens are provided in Table S2. All floral parts of each collected accession, such as involucral bracts, floral bracts, male and female flowers and seeds were studied (Fig. 2). The inflorescences for each accession were fixed in FAA for 24 – 48 h (5 ml of formalin: 5 ml of glacial acetic acid: 90 ml of ethyl alcohol) solution and then transferred to 70% ethanol for prolonged storage. Micro-morphological studies were carried out using a Leica M205C stereomicroscope. Images of dissected floral parts were taken. For SEM, seeds were directly mounted on aluminium stubs using double-sided carbon tape. Samples were then coated with gold for around 10 min at 15 kV using Emitech sputter coater and observed with Zeiss EVO 50 SEM (Carl Zeiss, Germany) at 15 – 20 kV acceleration voltage at 6 – 8 mm. Working Distance (WD) and images were captured directly using SmartView software.

Critical morphological studies were carried out for all samples collected as part of this study. Revisionary studies by Hooker (1893), Fyson (1919–1923), Zhang (1999), Ansari and Balakrishnan (1994, 2009) and several recently published research papers (Anto & Reshma, 2017; Biju et al., 2012, 2018; Chandore et al., 2019; Darshetkar et al., 2017, 2019; Francis et al., 2020; Khanna and Kumar, 2019; Manudev et al., 2017; Nampy et al., 2011; Naveen Kumar et al., 2017; Paithane et al., 2017; Rashmi & Krishnakumar, 2014; Shimpale et al., 2009; Shimpale & Yadav, 2010; Sunil et al., 2013; 2015; 2017; Sunil & Kumar, 2015; Swapna et al., 2012; Vivek et al., 2010) were followed for correct identification of *Eriocaulon* species. The nomenclature for seed surface type has been adopted after Lee et al. (2009).

Molecular studies

The ingroup consisted of 63 species of *Eriocaulon* while the outgroup consisted of eight species belonging to Paepalanthoideae and one *Xyris* sp. (Xyridaceae). The sequences for outgroup species were taken from the NCBI database (Table S2).

For molecular studies, two loci, one nuclear i.e. internal transcribed spacer region (ITS) and one chloroplast i.e. *trnL–F* intergenic spacer region were selected based on their proven utility in earlier molecular studies of the family (Andrade et al., 2010; Diaz Peãa, 2016; Trovó et al., 2013; Unwin, 2004). Genomic DNA was extracted using a modified CTAB method (Doyle & Doyle, 1987) or by QIAGEN DNeasy Plant Mini kit or by Bioneer Accuprep® Plant Genomic DNA extraction kit. The *trnL–F* region was either amplified as a single fragment using primers c/f or in two shorter fragments using primers c/d and e/f of Taberlet et al. (1991). The entire nuclear internal transcribed spacer region was amplified either with primer pair ITS 1 & 4 or ITS 5 & 4 (White et al., 1990) or to overcome the problem of fungal amplification in many species, ITS region was amplified using primers 17 SE and 26 SE (Sun et al., 1994). Partial ITS sequences were amplified using primers 17SE (Sun et al. 1994) and ITS2 (White et al. 1990) for difficult templates. Both loci were amplified using either AccuPower Taq® PCR PreMix from Bioneer or Emerald® GT PCR master mix. PCR products were purified either using QIAquick PCR purification kit from QIAGEN or by Polyethylene Glycol (PEG) precipitation (Sambrook and Russell, 2001). Sequencing reactions were carried out using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and purified by ethanol/EDTA precipitation according to the manufacturer's protocol. Dried pellets were suspended in 10 µL of Hi–Di formamide and run on ABI3100 Avant Genetic Analyzer as per the recommended protocol. All the sequences were submitted to the NCBI database (Table S2).

Phylogenetic analyses

The sequences were manually edited using BioEdit version 7.2.5 (Hall, 1999) and aligned with MUSCLE

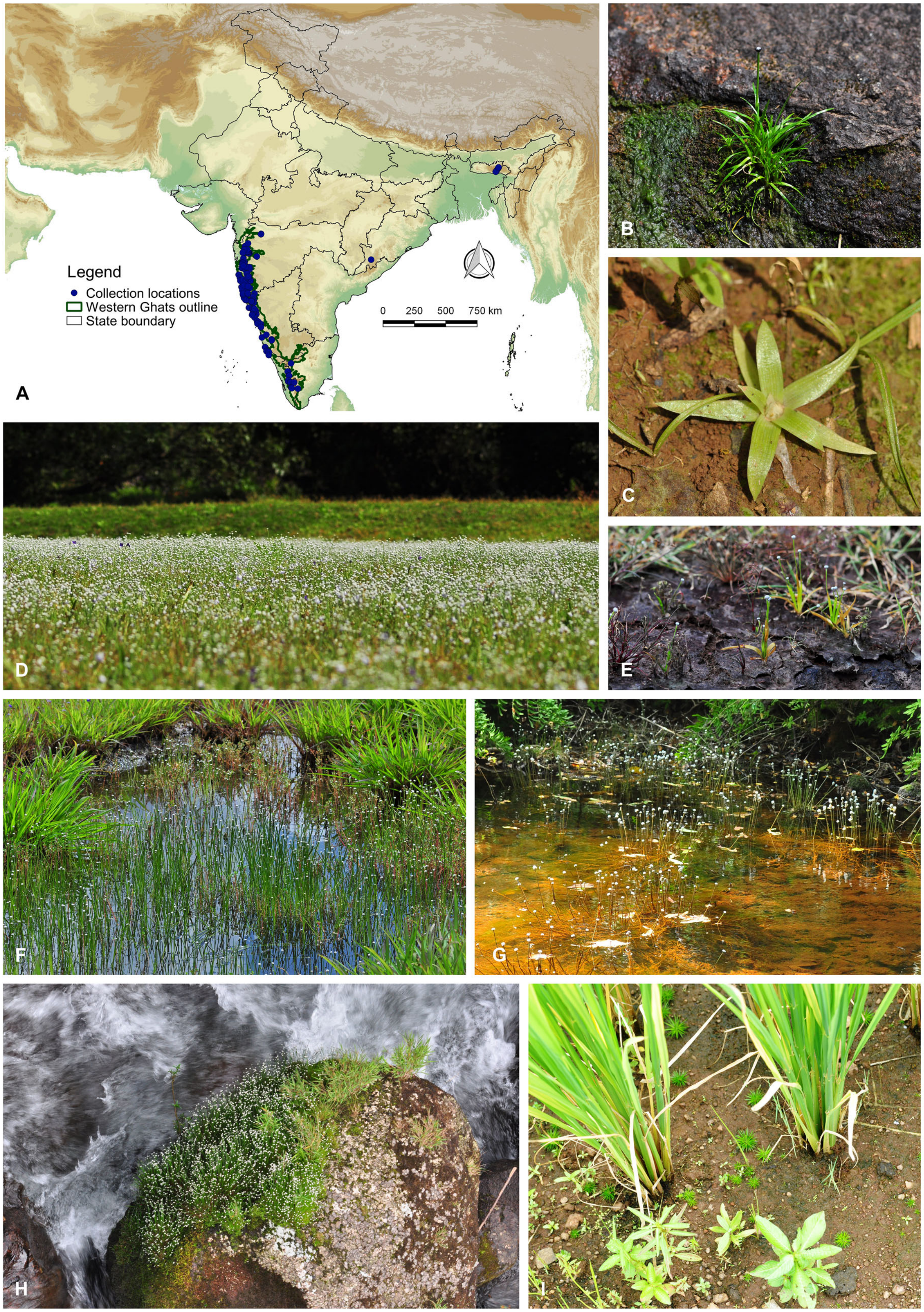


Fig. 1. A. Collection locations of *Eriocaulon* species during the years 2014–2018, B. to I. Habitats occupied by Indian *Eriocaulon* species.

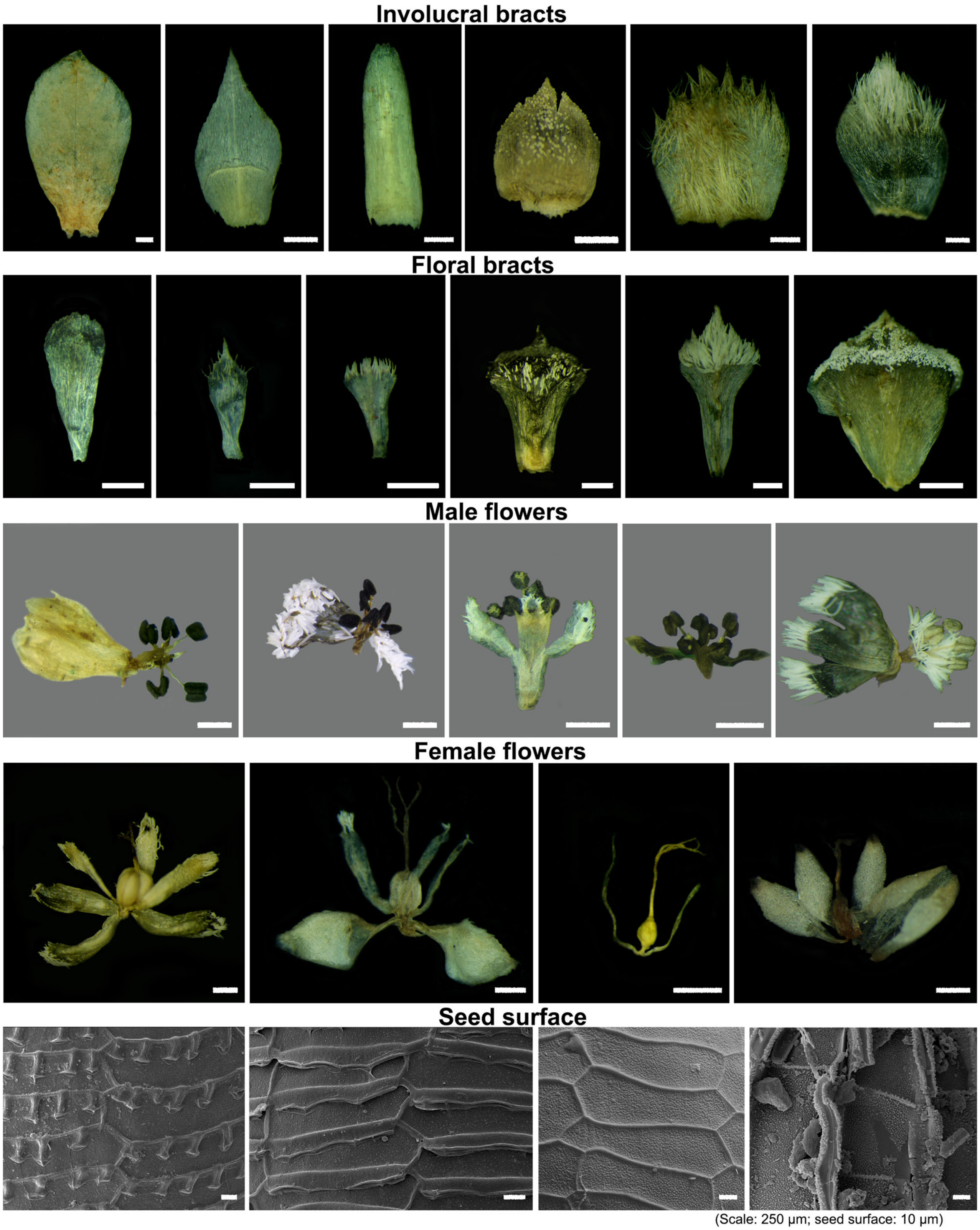


Fig. 2. Diversity in floral parts of the *Eriocaulon* species.

Table 1. Characters selected for ancestral state reconstruction.

Characters	Description
Anther colour (Black/White anthers)	Fyson (1919–1923,) grouped all white-anther species under section Leucantherae; also adopted by Zhang (1999).
Seed character	Sectional treatment by Ansari and Balakrishnan (2009) was based on seed characters. Zhang (1999) also used this character for cladistic analysis. In this study, shape of seed coat cells and types of seed appendages were considered and coded as multistate characters.
Glands on male petals (Presence/Absence)	Ruhland (1903) used this character to differentiate between Eriocauloideae and Paepalanthoideae. The former has glands on male petals while the latter is devoid of glands. Several Indian *Eriocaulon* species lack glands.
Stem (Presence/Absence)	Stem was considered of little taxonomic value. Most of the Indian species are acaulescent herbs while some of them have developed stems similar to Paepalanthoideae members. Despite being of little taxonomic value, there is a discrepancy in literature regarding this character (Ansari and Balakrishnan, 2009; Prajaksood et al., 2017).
Rootstock (Presence/Absence)	This underground stem-like portion was barely considered as an important character in the sectional treatment of Ansari and Balakrishnan (2009). However, in the last decade, several new species were reported from India considering rootstock as a distinguishing character (Manudev et al., 2017; Nampy et al., 2011; Naveen Kumar et al., 2017; Sunil et al., 2017).
Size of male petals (Equal/Unequal/Subequal)	Differences in the size of male petals were given importance by Fyson (1919–1923) and Zhang (1999) by raising a new section *Anisopetalae* for species having unequal male petals. Ansari and Balakrishnan (2009) considered it of "little value in differentiation, due to large number of intermediate forms".
Male sepals (Fused/Free)	Sectional treatment by Ansari and Balakrishnan (2009) considered this of prime importance along with the seed character.
Hairiness of involucral and floral bracts (Glabrous/Hairy)	Fyson (1919–1923) considered the hairiness of bracts as differentiating character to delimit the sections (section *Scariosae*). Ansari and Balakrishnan (2009) considered hairiness of bracts of secondary importance to delimit the species.

(Edgar, 2004). The best partitioning scheme and model of sequence evolution was decided using PartitionFinder 2 (Lanfear et al., 2017). Maximum Likelihood (ML) analyses were performed using IQ–TREE v. 1.6.7 for all three datasets (i.e. ITS, *trnL–F* and combined). For Bayesian inference, both independent and combined analyses were carried out. For the ITS region, data was partitioned into three parts, ITS1, 5.8 s and ITS2 region. For the *trnL–F* region, data were partitioned into intron and spacer region. Both the datasets were partitioned based on manual annotations and NCBI BLAST results. For the ITS and the *trnL–F* region, Bayesian analysis was performed using MrBayes version 3.2.6 (Ronquist et al., 2012), for 40,00,000 generations, with two independent runs of four chains, sampling every 1000 generations. The output parameters were checked in Tracer v1.6 (Rambaut et al., 2014) to assess convergence. For the combined analyses both the datasets were concatenated using the software TaxonDNA (Vaidya et al., 2011) and the analysis was run for 55,00,000 generations. The first 25% of the sample was discarded as burn-in and the rest were used to calculate posterior probability. Trees obtained from ML and Bayesian analysis were visualized using Figtree v. 1.4.3 (Rambaut, 2017).

Ancestral state reconstruction

Ten morphological characters were selected for this study based on the literature review (Ansari & Balakrishnan, 1994, 2009; Fyson, 1919–1923; Zhang, 1999), herbarium records, and the characters observed during this study. The characters chosen were anther colour, seed appendages and seed coat cell shape, hairiness of bracts (involucral and floral), size of male petals, glands on male petals, fusion of male sepals, presence of stem and rootstock and the rationale behind

the selection of these characters has been presented in Table 1.

Other characters which exhibited intraspecific variation were not considered.

Characters of outgroup species were coded from the available literature (Moldenke, 1957; Trovó et al., 2013). Selected characters were coded as either binary or multistate for state reconstruction (Table S3). The evolutionary history of each character was reconstructed on to a 50% majority rule tree from Bayesian analysis using ML and Bayesian inference. The ML reconstructions were carried out using Mk1 (Markov 1 parameter) model (Pagel, 1999; Schluter et al., 1997) as implemented in Mesquite v. 3.31 (Maddison and Maddison, 2017). Ancestral state reconstruction using Bayesian analyses were carried out in BayesTraits V.3.0.1. The analysis was run for 1.05×10^6 generations sampling every 1000 generations out of which the initial 500,000 iterations were discarded as burn-in. Some nodes were fossilized to test if the value of one-character state is significant (Pagel et al., 2004). Convergence and effective sample size (ESS) were checked in Tracer v 1.6 (Rambaut et al., 2014). Nodes were reconstructed using AddMRCA command (Pagel et al., 2004). The cut-off posterior probability value to denote ambiguous reconstructions was 0.95.

Results and discussion

Morphological studies

From 552 accessions collected during this study from different parts of India, a total of 66 species were identified. Out of 86 species reported from the Western Ghats, 61 species were included in this study (Table S4). Five north-east Indian endemic species were also included (Table S4). Two new species, viz. *Eriocaulon parvicephalum* Darsh., R.K.Choudhary, Datar & Tamhankar and *Eriocaulon karaavalense* Darsh., R.K.Choudhary, Datar & G.R.Rao were identified from the collected accessions (Darshetkar et al., 2017, 2019).

Earlier, Zhang (1999) reported various types of seed appendages for East Asian *Eriocaulon* species, however, during this study seed surface characters of Indian species were recorded for the first time using SEM imaging. For a few species, these could not be studied as the seeds were not available due to the presence of immature inflorescences in the collected specimens. Seed SEM images of 23 species were presented in our earlier studies (Darshetkar et al., 2017, 2019). Characters of 30 species have been shown in Figs 3 and 4. Table 2 reports the seed surface characters of 55 *Eriocaulon* species varying from reticulate (Fig. 3: 22), reticulate–foveate (Fig. 4: 6), ruminate (Fig. 4: 12), granulate (Fig. 4: 2), tuberculate (Fig. 4: 20), smooth (Darshetkar et al., 2017, Fig. 4: h), foveate (Fig. 4: 24) and pusticulate (Fig. 4: 32) types.

Recollection of *Eriocaulon collettii*

Eriocaulon collettii is only known from Meghalaya in India. The species occupies marshy habitat, is sometimes submerged in water and is characterized by unequal female petals and setiform seed appendages arising from radial walls (Fig. 5). During the present study, the species was recollected (see Supplementary Appendix 1) after more than four decades of its discovery (Naik, 1974).

Molecular studies

During the present study, 123 new sequences were generated. ITS sequences of 60 species and *trnL–F* sequences of 63 species were included in the analysis.

The ITS region was the most difficult for amplification and exhibited problems such as endophytic fungal ITS amplification and paralogous copies of the region were encountered in amplification. However, primers designed for *Sorghum* (Sun et al., 1994) proved to be useful. The ITS region varied from 645–695 bp while *trnL–F* varied from 823–954 bp. Only partial ITS region (ITS1) could be amplified for *E. madayiparense*, *E. gopalakrishnanum*, *E. heterolepis* and *E. apetalum*, length of which varied from 349–356 bp. Also, only *trnL* intron could be amplified for *E. robustum*, *E. trilobum* and *E. heterolepis* and the length varied from 557–572 bp. External gaps were coded with question marks. The total aligned length of ITS region was 876 bp while that of *trnL–F* region was 1242 bp. ITS and *trnL–F* analyses included sequence data of 69 and 72 species, respectively (including outgroup species). *E. minutum*, *E. maharashtrense* and *E. leucomelas* could not be included due to bad sequence quality. The total length of the combined dataset was 2118 bp.

Phylogenetic studies

The best-fitting models suggested by IQtree software were GTR + F + I + G4, TVM + F+G4 and TIMe + R3 for ITS, *trnL–F* and combined analyses respectively. Partition Finder 2 suggested GTR + I + G for ITS1, ITS2 regions and K80 + G for 5.8 s region. For *trnL–F* region, K81uf + G (spacer) and TVM + G (*trnL*) models were selected by Partition Finder 2. These models were implemented by using different rate and shape parameters in MrBayes software. Trees obtained from

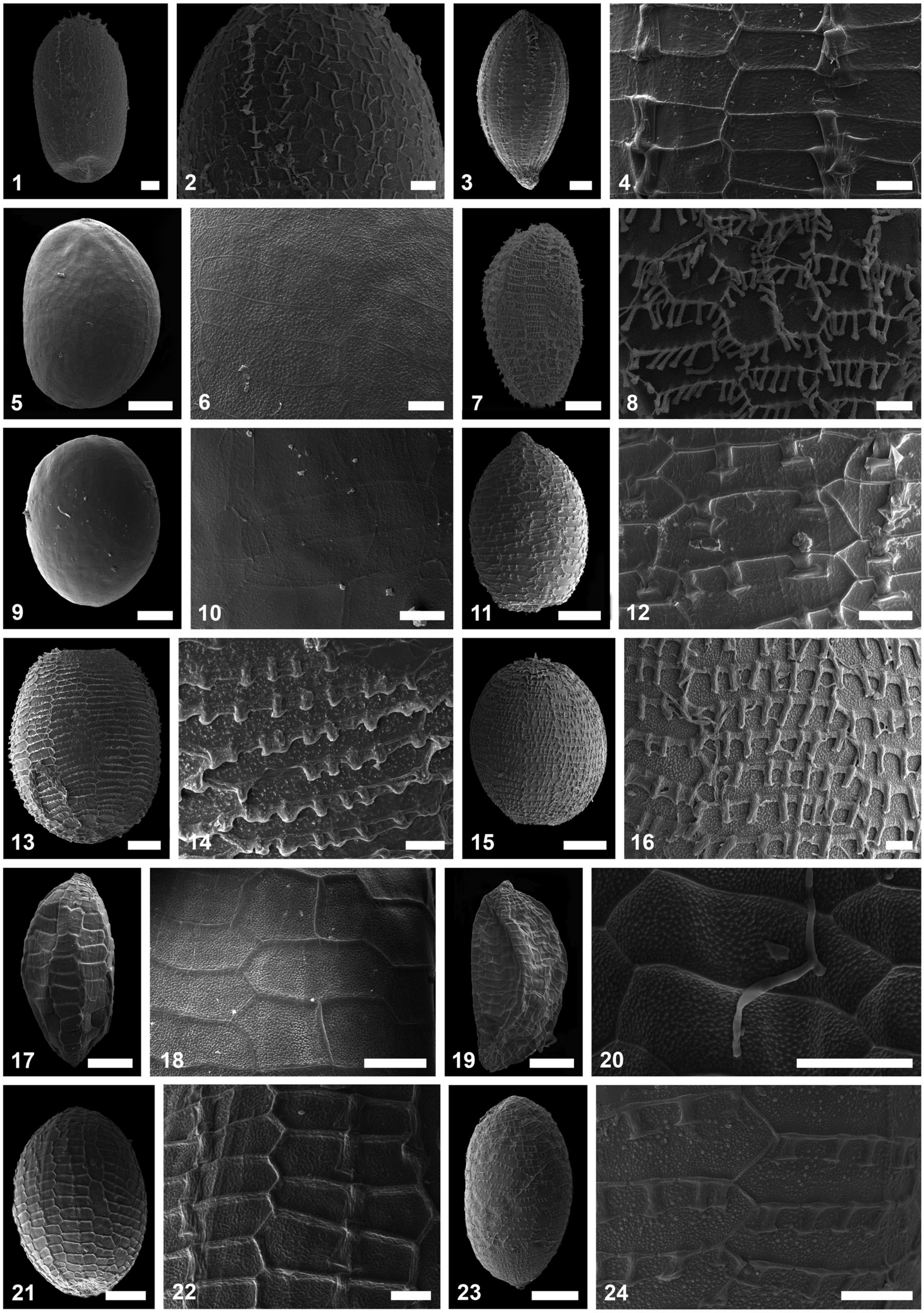
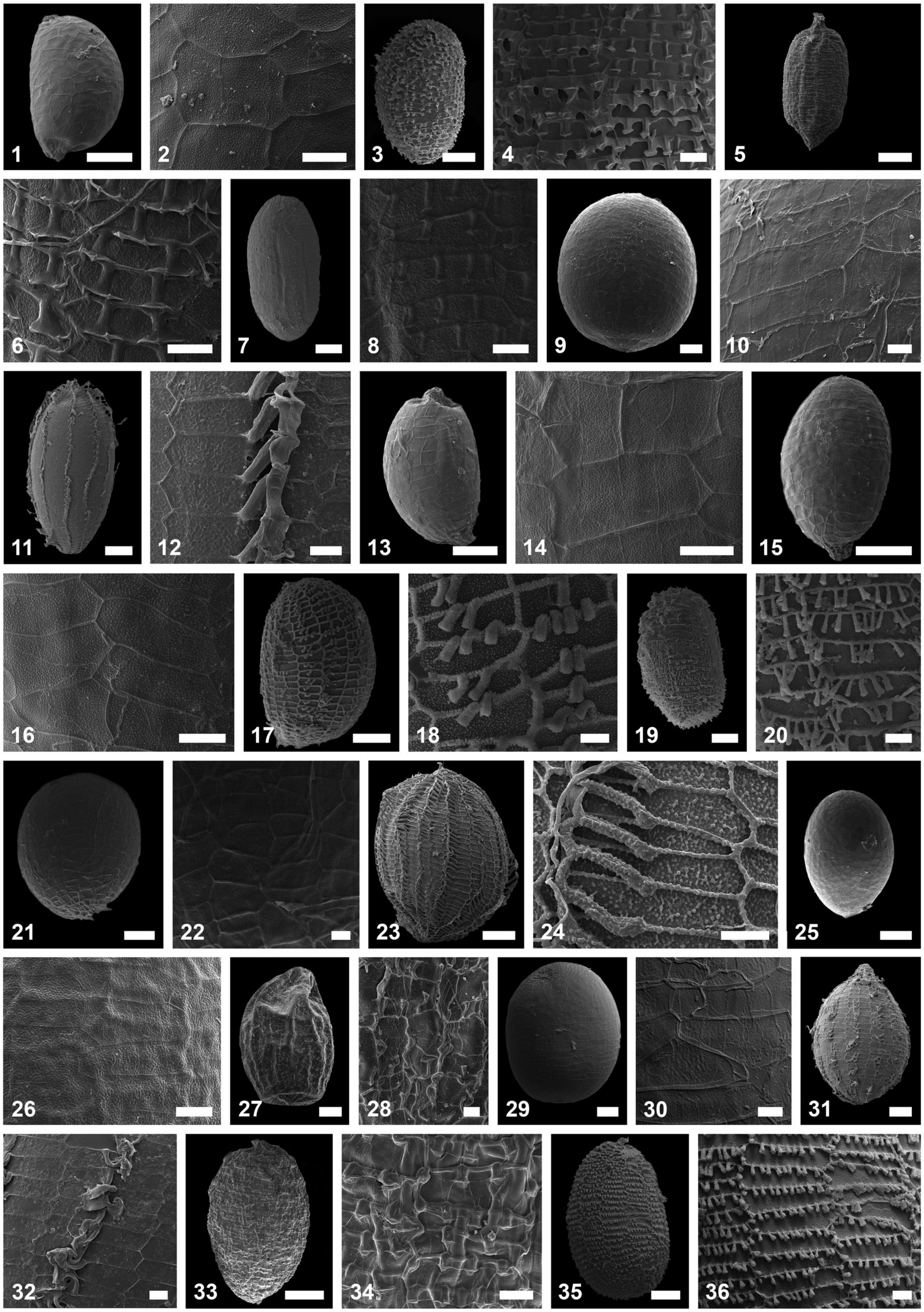


Fig. 3. SEM images: 1 & 2. *E. alpestre*, 3 & 4. *E. anshiense*, 5 & 6. *E. cinereum*, 7 & 8. *E. collettii*, 9 & 10. *E. dalzellii*, 11 & 12. *E. duthiei*, 13 & 14. *E. setaceum*, 15 & 16. *E. sharmae*, 17 & 18. *E. minutum*, 19 & 20. *E. rajendrababui*, 21 & 22. *E. thwaitesii*, 23 & 24. *E. gopalakrishnanum.* Scale bars: Seed images = 100 µm, Seed surface = 20 µm.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36

independent analyses of ITS and *trnL–F* regions were largely congruent albeit showing slight differences (Figures S1 and S2). Both the analyses yielded three major clades similar to the tree obtained from the combined analysis. However, placement of some of the species mentioned below was incongruent in the trees obtained from both the loci.

A. *Eriocaulon conicum*: According to ITS dataset (Figure S1), *E. conicum* appeared sister to the clade of *E. cheemenianum*, *E. karaavalense* and *E. odoratum* (BP = 96, PP = 1), while based on *trnL–F* data (Figure S2), it was sister to *E. baramaticum* and *E. duthiei* and the relationship was unresolved (BP = 100, PP = 1). However, in both the trees it was placed under clade I. If morphological characters are to be considered, the grouping obtained from *trnL–F* dataset appears more appropriate than that of the ITS dataset.

B. *Eriocaulon epedunculatum*: In both the trees (Figures S1 and S2), this species was placed under clade III. However, in ITS tree it was sister to *E. stellulatum* (BP = 100, PP = 1) while in *trnL–F* tree it was placed next to *E. brownianum* but the placement was unresolved with very low BP and PP values. The species is morphologically allied to *E. stellulatum* which was aptly exhibited by ITS tree as the two species were resolved as sister.

C. *Eriocaulon quinquangulare*: According to ITS tree, the species was placed under clade II as sister to *E. cinereum* and *E. redactum* (BP = 99, PP = 1). In *trnL–F* tree, it was placed under clade I, sister to *E. trilobum* (BP = 100, PP = 1). Here, the placement shown by *trnL–F* tree seems more appropriate.

D. *Eriocaulon mitophylum*: In ITS tree, it was placed under clade II as sister to *E. madayiparense* (BP = 98, PP = 1) while in *trnL–F* tree, both the species were placed under clade I but the relationship was unresolved. *E. mitophylum* is a white anther species but both the loci failed to group it with other white anther species.

E. *Eriocaulon palghatense*: In both the trees (Figures S1 and S2), *E. palghatense* was placed under clade I. However, it appeared sister to *E. parvicephalum* in ITS tree (BP = 100, PP = 1), but to *E. cheemenianum* in *trnL–F* tree (BP = 99, PP = 1). This could be because of a large deletion in *trnL–F* region of *E. palghatense*.

The differences in topologies exhibited by ITS and *trnL–F* loci in Eriocaulaceae could be due to gene flow or incomplete lineage sorting (Diaz Peña, 2016). It is easier to detect hybridization events when data from both the parents are available. In this study, we used one nuclear and one plastid marker. Plastid markers exhibit uniparental inheritance, and nuclear ones exhibit biparental inheritance. However, the chances of masking the biparental inheritance due to concerted evolution in nuclear markers cannot be neglected (Fuertes et al., 1999; Okuyama et al., 2005; Wendel et al., 1995). Also, the biparental inheritance of plastids has been reported for many monocot families (Corriveau & Coleman, 1988; Yao et al., 1994; Zhang & Liu, 2003). However, further studies are required to trace the cause behind different topologies obtained for ITS and *trnL–F* loci in many Eriocaulaceae studies.

In the present study, the combined dataset resulted in a more resolved phylogeny than those derived from individual loci. Also, the combined tree generated using ML and Bayesian methods yielded similar topologies and exhibited three major clades of Indian *Eriocaulon* species (Clades I, II and III, Fig. 6). Hence, the results of the combined tree are presented and discussed here (Fig. 6).

The combined tree identified *Eriocaulon alpestre* Hook.f. & Thomson ex Körn. as the first branching lineage, separate from all the three major clades. The species was placed in subgenus *Spathopeplus* Körn., section *Apoda* (Satake) Zhang (1999). It has a wider distribution in South East Asia and is the only Indian species having fused female sepals. Owing to this apomorphy, Ansari and Balakrishnan (1994, 2009) placed it in Sect. I, as the sole representative of the section. We believe the placement of the species and its phylogenetic relationships will be comprehensible only after adding more species from section *Apoda* (Zhang, 1999).

The first clade (Clade I, Fig. 6) consisted of the species belonging to sections III, IV, VI, VII, VIII, IX and X proposed by Ansari and Balakrishnan (1994, 2009) resolved in five subclades (BP = 100, PP = 1). In the first subclade, *Eriocaulon baramaticum*, *E. duthiei*, *E. conicum* and *E. robustum* appeared sister species with strong support (BP = 100, PP = 1). They were placed in sections X, VI and VII respectively due to differences in seed appendages. However, they are allied in having acuminate oblong-lanceolate floral bracts and linear-elliptic female sepals. *E. conicum* differs from the

Fig. 4. SEM images: 1 & 2. *E. elenorae*, 3 & 4. *E. heterolepis*, 5 & 6. *E. kolhapurense*, 7 & 8. *E. koynense*, 9 & 10. *E. leucomelas*, 11 & 12. *E. madayiparense*, 13 & 14. *E. maharashtrense*, 15 & 16. *E. margaretae*, 17 & 18. *E. nepalense*, 19 & 20. *E. pykarense*, 21 & 22. *E. pectinatum*, 23 & 24. *E. rhodae*, 25 & 26. *E. redactum*, 27 & 28. *E. robustum*, 29 & 30. *E. idukkinaum*, 31 & 32. *E. sexangulare*, 33 & 34. *E. stellulatum*, 35 & 36. *E. trilobum*. Scale bars: Seed images = 100 µm, Seed surface = 20 µm.

Table 2. Seed surface characters of the studied species.

Species	Surface
E. mitophylum	Faintly reticulate
E. rhodae	Foveate
E. breviscapum, *E. cheemenianum*, *E. cinereum*, *E. conicum*, *E. cristatum*, *E. dalzellii*, *E. elenorae*, *E. gopalakrishnanum*, *E. heterolepis*, *E. karaavalense*, *E. leucomelas*, *E. maharashtrense*, *E. margaretae*, *E. minutum*, *E. nepalense*, *E. odoratum*, *E. palghatense*, *E. parvicephalum*, *E. parviflorum*, *E. rajendrababui*, *E. redactum*, *E. richardianum*, *E. setaceum*, *E. sivarajanii*, *E. stellulatum*, *E. trilobum, E. xeranthemum*	Granulate
E. sexangulare	Pusticulate
E. alpestre, *E. fluviatile aff*, *E. thwaitesii*, *E. vasudevanii*	Reticulate
E. achiton, *E. anshiense*, *E. apetalum*, *E. eurypeplon*, *E. kolhapurense*, *E. koynense*, *E. sedgwickii*, *E. tuberiferum*	Reticulate-foveate
E. baramaticum, *E. brownianum*, *E. duthiei*, *E. madayiparense*	Ruminate
E. cuspidatum, *E. fysonii*	Smooth
E. collettii, *E. pykarense*, *E. robustobrownianum*, *E. robustum*, *E. sharmae*, *E. truncatum*	Tuberculate

former two in having three male and female sepals. *E. baramaticum* is predominantly similar to *E. duthiei* but lacks seed appendages and exhibits variation in the number of sepals. *E. robustum* is characterized by the most robust habit of the genus found in India. It appeared to be sister to the clade of *E. duthiei*, *E. baramaticum* and *E. conicum* with strong statistical support (BP = 98, PP = 0.9). However, morphologically *E. robustum* varies with these in having rootstock, peduncles up to 65 cm long, unequal male and female petals and setiform appendages from all radial walls and share similarity of acute, glabrous involucral bracts.

The next subclade consisted of *Eriocaulon cherrapunjianum*, *E. nepalense*, *E. collettii*, *E. pykarense* and *E. setaceum* (BP = 100, PP = 1) and resolved as sister to the clade comprising *E. heterolepis*, *E. parviflorum*, *E. richardianum* and *E. kolhapurense*. It is difficult to discuss the placement of these species as they share no distinct morphological characters. Additional species as well as a few more loci need to be included in the analysis to resolve relationships between these species.

Eriocaulon karaavalense, *E. parvicephalum*, *E. palghatense*, *E. odoratum* and *E. cheemenianum* were resolved as a clade (BP = 62, PP = 0.62). In agreement with our previous studies (Darshetkar et al., 2017, 2019), *E. parvicephalum and E. palghatense*, were resolved as a clade. *Eriocaulon gopalakrishnanum*, *E. longicuspis* and *E. trilobum* appeared to be successive sister taxa to the above subclade (BP = 90, PP = 0.92).

Eriocaulon sivarajanii, *E. xeranthemum* and *E. vasudevanii* that all possess characteristic erect and spreading involucral bracts were resolved as a clade (BP = 100, PP = 1) in this study. The relationship of *E. cristatum* and *E. truncatum* are not resolved. Both species belong to section VII of Ansari and Balakrishnan (1994, 2009), characterized by non-setiform seed appendages. However, *E. cristatum* possesses appendages on vertical walls (vs. on horizontal walls in other species) while *E. truncatum* has vertically elongated seed coat cells (vs. transversely elongated in other species of the section). These two are the only Indian species exhibiting such unique seed features, probably due to which the placement appeared unresolved. Adding the species having similar morphological features from other geographic regions might help in resolving the placement of these species.

In congruence with morphology, most of the section IX and X species, viz. *E. elenorae*, *E. margaretae*, *E. maharashtrense*, *E. minutum* and *E. rajendrababui* were resolved within the fifth subclade (BP = 92, PP = 0.81). All these species lack seed appendages and are characterized by black anthers. However, these two sections were earlier segregated based solely on the shape of seed coat cells. Species of section IX are characterized by isodiametric seed coat cells while section X species exhibit transversely elongated seed coat cells. Our data do not corroborate this treatment. The character of seed coat cell shape appears to be homoplastic.

Clade II consisted of three subclades (BP = 72, PP = 0.72) and species belonging to sections VI, VII, X, XI and XII. The first subclade comprised all of the species possessing white anthers except for *E. mitophylum* (possesses white anthers). *Eriocaulon quinquangulare* (black anthers) was resolved as sister to this subclade (BP = 64, PP = 0.74). The first subclade consisted of *E. breviscapum*, *E. dalzellii*, *E. cookei*, *E. idukkianum*, *E. tuberiferum*, *E. pectinatum*, *E. talbotii*, *E. ritchieanum*, *E. cinereum* and *E. redactum*. Though a white anther species, *E. tuberiferum* was placed in section XI due to thickenings on the seed coat cell walls. *E. idukkianum*, a

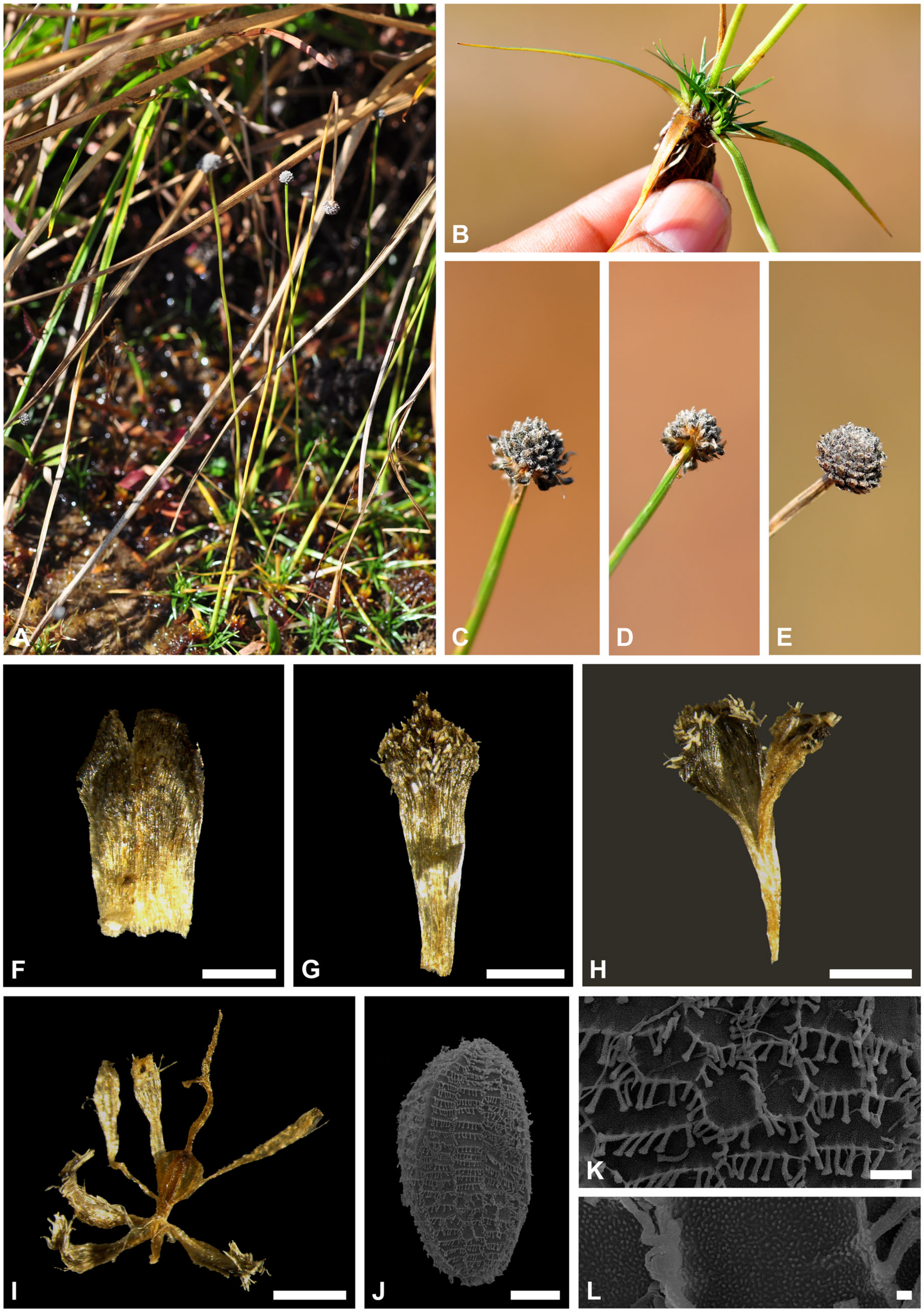


Fig. 5. *Eriocaulon collettii* Hook.f. A. Habit & Habitat, B. Leaves, C. to E. Heads, F. Involucral bract, G. Floral bract, H. Male flower, I. Female flower, J. Photomicrograph of seed, K. Seed appendages, L. Seed surface: tuberculate. Scale bars = F. to I. 500 μm, J. 100 μm, K. 20 μm, L. 2 μm.

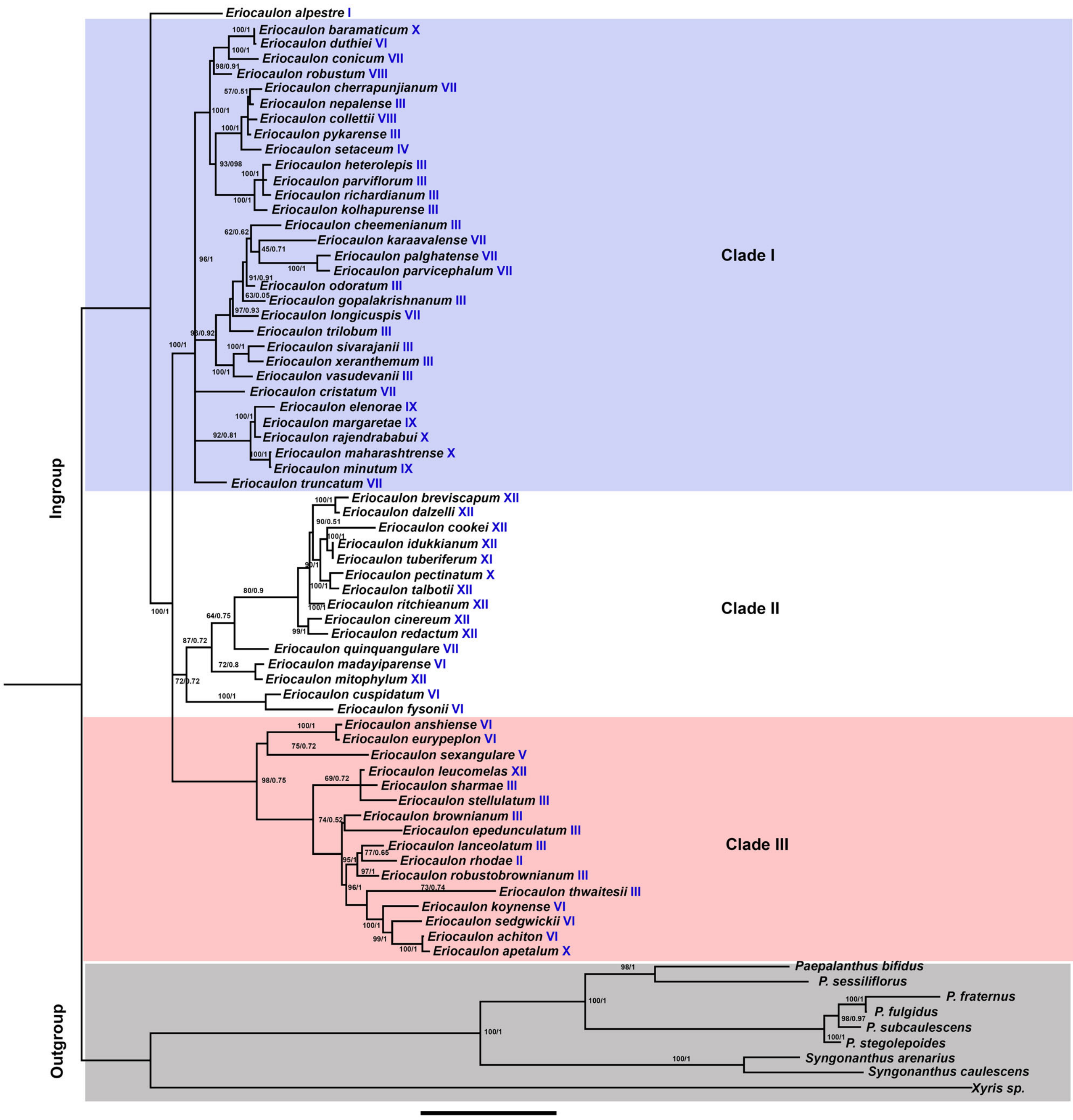


Fig. 6. The strict consensus tree obtained from Bayesian analysis of combined ITS and *trnL–F* dataset. Numbers indicate bootstrap (obtained from ML analysis) and Posterior probability values. Numbers in Roman (blue) are the sections proposed by Ansari and Balakrishnan (2009).

recently described species and no molecular differences were observed (see discussion later).

E. pectinatum (sect. X) has black anthers but still grouped with the white anther species. It shares characters such as the presence of unequal petals and seeds devoid of appendages, which are observed in white anther species. In the next subclade, *E. madayiparense* is morphologically allied to *E. eurypeplon* (resolved in Clade III), however, based on our data it is resolved as sister to *E. mitophylum* (BP = 72, PP = 0.8). The two species do not share any similarities in morphology. *Eriocaulon cuspidatum* and *E. fysonii* that possess cuspidate leaf apex appear to be sister species (BP = 100, PP = 1). These two species are morphologically allied but differ in the shape and size of involucral bracts.

Clade III consisted of species belonging to sections II, III, V, VI, X and XII and five subclades were identified. *E. achiton* and *E. apetalum* were earlier placed in different sections merely due to the differences in seed morphologies but they are resolved as sister taxa (BP = 100, PP = 1) and possess identical morphologies with the exception of the seed appendages: *E. achiton* is characterized by setiform appendages while *E. apetalum* lacks such appendages; however, both species are characterized by reticulate-foveate seed surfaces (Table 2).

In congruence with the morphological data, *Eriocaulon anshiense* and *E. eurypeplon* were resolved as sister species (BP = 100, PP = 1). These species are morphologically allied but exhibit differences. The former is characterized by long acuminate female sepals and beaked seeds while the latter possesses acute or obtuse sepals and non-beaked seeds. *Eriocaulon sexangulare* was resolved as sister to the clade of *E. anshiense* and *E. eurypeplon* (BP = 75, PP = 0.72). The species is similar to these two in most features except that the male flowers have closed, tubular spathes.

The second subclade in clade III is composed of *E. leucomelas*, *E. sharmae* and *E. stellulatum* (BP = 69, PP = 0.72). Relationships within this subclade are unresolved. *Eriocaulon sharmae* is morphologically allied to *E. robustobrownianum* in having strikingly acuminate floral bracts and similar male and female flowers while *E. stellulatum* is morphologically allied to *E. epedunculatum* in almost all floral characters except its peduncles and in its seed appendages. These species also resolved within clade III but in different subclades. *Eriocaulon leucomelas,* a white anther species, shares no similarity with those in subclade II.

The next subclade combined *Eriocaulon brownianum* and *E. epedunculatum* which are morphologically not related as per the earlier circumscriptions (Ansari & Balakrishnan 1994, 2009). These two species appeared to be sister, however, with moderate to poor statistical support (BP = 74, PP = 0.52). *E. epedunculatum* is the only Indian species lacking peduncles. *E. escape* B.F.Hansen and *E. pseudoescape* Prajaksood & Chantar. from Thailand are also known to be devoid of peduncles. So, adding more related species might help to understand their grouping.

The last subclade combined all *Eriocaulon* species characterized by the presence of two sepals in both male and female flowers (BP = 73, PP = 0.74) except *E. thwaitesii* (Sect. III) which possesses two or three sepals in both male and female flowers. The species exhibiting this character were grouped under section VI. However, this section is not supported by molecular data as the species are dispersed throughout the tree. *Eriocaulon sedgwickii* and *E. koynense* were also placed in section VI. These two were resolved as sister to the clade of *E. apetalum* and *E. achiton* (BP = 99, PP = 1; BP = 100, PP = 1). *Eriocaulon sedgwickii* is known to have villous involucral and floral bracts while *E. koynense* is the only Indian species having dimorphic bracts.

Ancestral state reconstruction

Character reconstructions carried out using Maximum Likelihood and Bayesian methods were congruent for all 10 selected morphological traits. Morphological evolution of all these characters is discussed below in light of the results obtained using molecular data.

Anther colour. Anther colour was considered an important character in the morphological classifications proposed by Fyson (1919–1923), Zhang (1999) and Ansari and Balakrishnan (1994, 2009). Fyson grouped all white-anther species under the section *Leucantherae*. Zhang (1999) also adopted this section from Fyson while classifying East Asian *Eriocaulon* species. Ansari and Balakrishnan (1994, 2009) placed white anther species under two sections XI and XII. Leach (2017) also used anther colour character while preparing a taxonomic key for Australian *Eriocaulon* species. Based on current sampling, our results identify black anthers as plesiomorphic while white anthers to have evolved independently at least twice in clade II and clade III (Fig. 7*a*, S4). Anther colour character thus appears to be homoplastic (Fig. 7*a*, S4) and the sections proposed in earlier studies based on this character are not supported.

Seed appendages. Nair (1987) recognized the importance of seed surface characters in identifying *Eriocaulon* species. He studied 19 species for surface characters and found seed coat characters to be of diagnostic value in most species. Zhang (1999) also identified it as an important character and recognized five types of projections (appendages). Ansari and Balakrishnan (1994, 2009) also emphasized the importance of seed morphology in identifying Indian *Eriocaulon* species and treated them under 12 sections mainly based on seed coat surface characters. The projections on seeds were referred as tertiary protuberances by Zhang (1999) and seed appendages by Ansari and Balakrishnan (1994, 2009). They are either setiform, ribbon-shaped, or without any appendages. The ontogenic development of *Eriocaulon* seeds has been studied comprehensively by Giulietti et al. (1987) and Phillips (1994). The seed testa has two integuments and the outer layer of the outer integument is made up of small

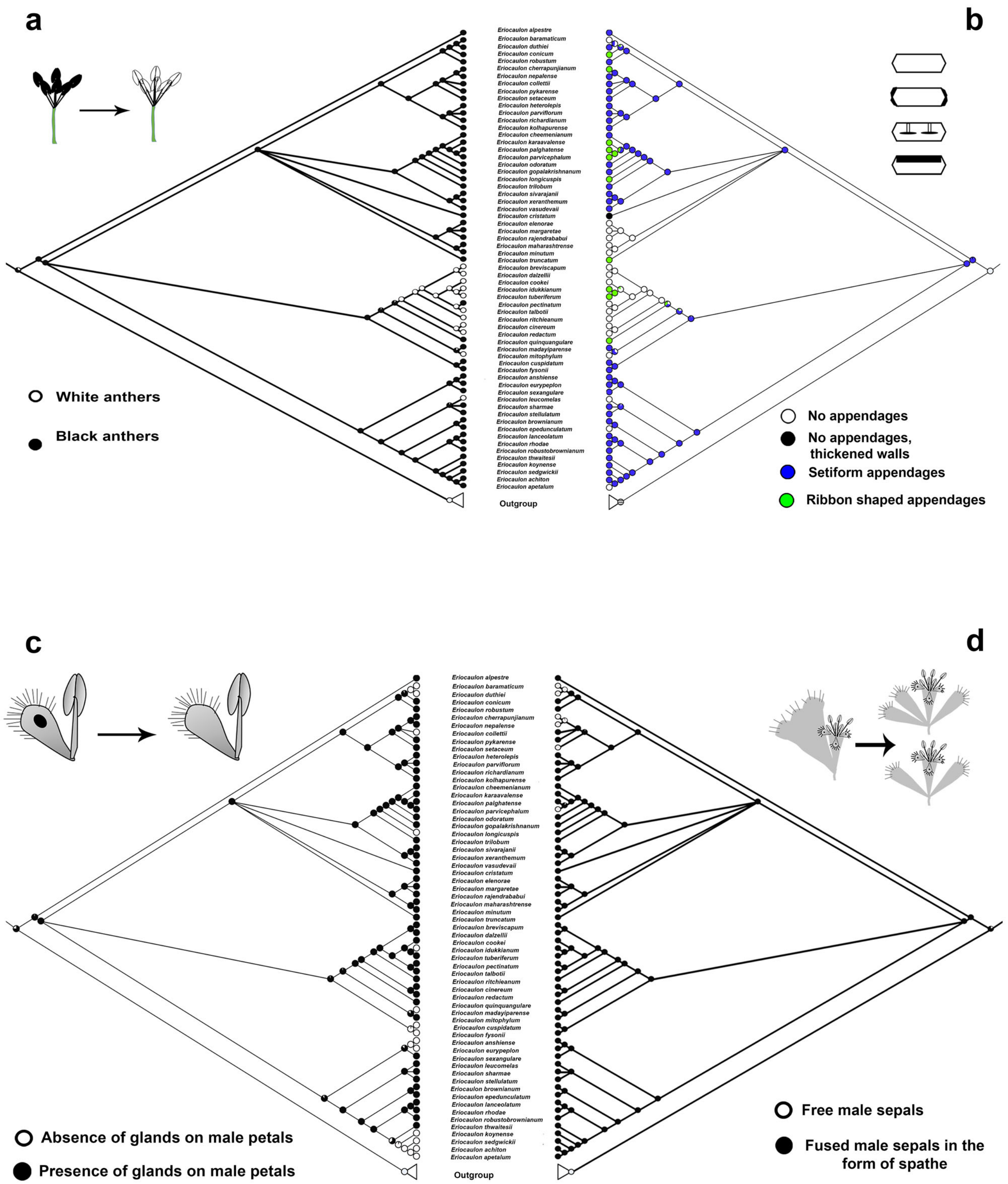


Fig. 7. Distribution of characters onto a Bayesian tree: a. Anther colour. b. Types of seed appendages, c. Presence or absence of glands on male petals, d. Fused or free male sepals.

and thin cells which remains intact in some species and forms a diaphanous layer. However, in most of the species, the outer layer degenerates and exposes the inner layer of the outer integument. The inner layer has cells (either referred to as anticlinal walls or transversely elongated or isodiametric cells) which have thickenings

(that may be restricted to particular walls) that develop projections (Giulietti et al., 1987; Leach, 2017; Phillips, 1994). The presence of Setiform seed appendages is resolved as the ancestral state for the genus (Fig. 8*b*). Several species in all three clades lack appendages and different types of appendages have evolved independently in all the three clades (Fig. 7*b*, S5). Ribbon-shaped appendages are distributed in clades I and II. *E. cristatum* is the only species having cell thickenings without any appendages, in which the character must have evolved independently. Our study suggests transversely elongated cells could be the ancestral condition for the genus and other cell shapes like isodiametric and rectangular have evolved in clade I (Fig. 8*a*, S8).

It is hypothesized that seed appendages play a vital role in seed dispersal of *Eriocaulon* species which is carried out either by wind and/or water (Stützel, 1998). Leach (2017) stated that these appendages increase the diameter and surface area of seeds which allows flotation and also helps in anchoring the seeds to the substrate. However, he also pointed out that despite the lack of such seed ornamentations, species such as *E. cinereum* exhibit wide distributions. Ansari and Balakrishnan (1994, 2009) placed species without appendages under sections IX, X and XII. All white anther species found in India (except *E. tuberiferum*) lack seed appendages. In our study, most of the section IX and XI species form a monophyletic group, except *E. pectinatum* and *E. apetalum*. Some Indian species differ only in seed appendages, viz. *E. achiton* and *E. apetalum*, *E. baramaticum* and *E. duthiei*. We observed intraspecific variations in the seed coat surface of *E. duthiei*. Some species complexes were identified which exhibit similar floral and seed morphology but that differ in just seed projections.

Conclusively, our analysis suggests the appearance of seed appendages (degeneration of outer layer of an outer integument) and their setiform feature could be the plesiomorphic states for the genus. Presence or absence of the appendages and their types are of little value for grouping of Indian *Eriocaulon* species (Fig. 7*b*, S8).

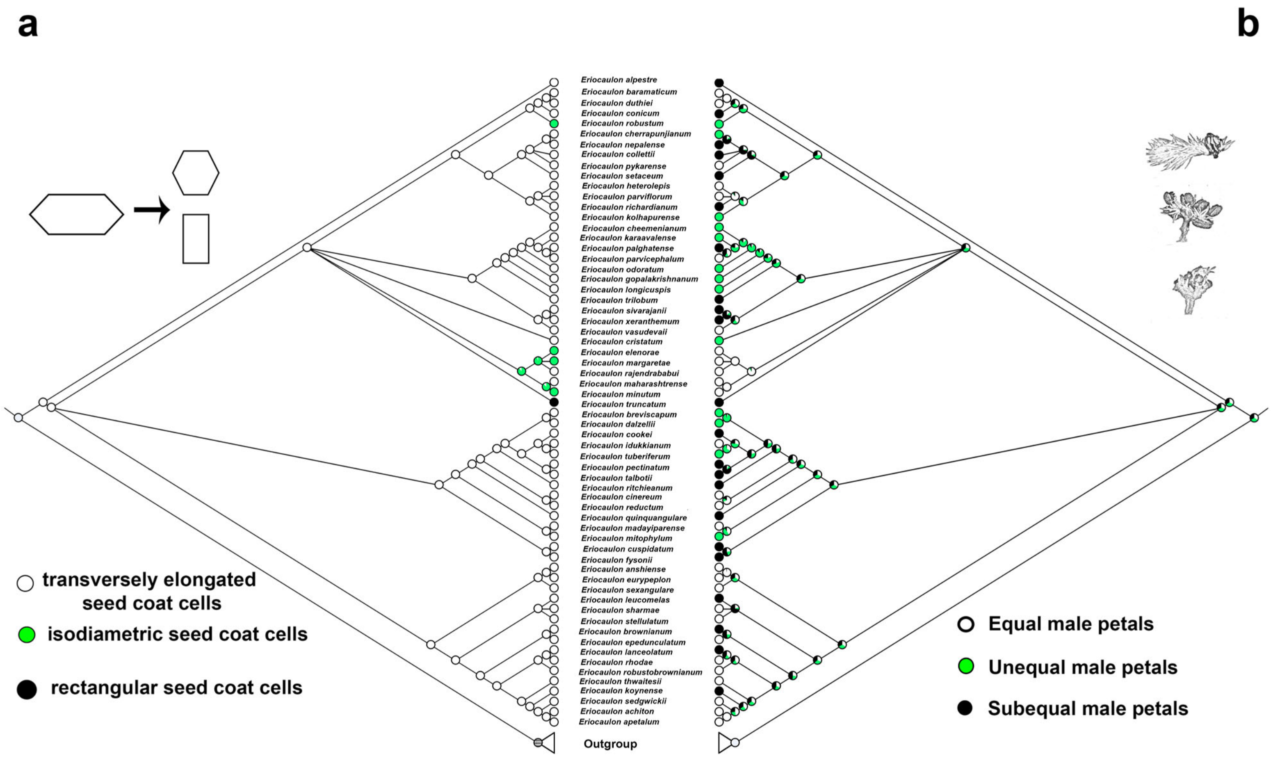
Glands on male petals. Our study suggests the presence of glands on male petals as an ancestral character for the genus *Eriocaulon*, in accordance with the earlier studies (Ruhland, 1903; Rosa and Scatena, 2007). The absence of glands could be seen in some species of clade I (Fig. 7*c*, S6). We did not observe any intraspecific variation in this character during this study. Few species of *Eriocaulon* (e.g. *E. duthiei*, *E. baramaticum*, *E. cherrapunjianum*) are devoid of petal nectaries and it will be interesting to study their pollination system which could probably be abiotic and without any involvement of a pollinator.

Fused or free male sepals. Most of the Indian *Eriocaulon* species are trimerous and have three sepals in male flowers either fused or free. The sepals are fused on the abaxial side and fused at the base and split open at the apex. *Eriocaulon sexangulare* is one of three species (along with *E. peninsulare* and *E. longifolium* (=*E. willdenovianum* Moldenke)) that exhibit a completely closed tubular spathe and it is the only species exhibiting this trait that was included in the analysis presented in this paper. We coded this species as possessing fused sepals.

The analysis indicates that free sepals have evolved several times within the group (Fig. 7*d*, S7). Species with free sepals may be di- or trimerous.

Size of male petals. Flowers of *Eriocaulon* species are minute and sometimes it is hard to find the petals of male flowers for critical taxonomic observation. However, three types can be defined based on their relative size, viz. equal, subequal and unequal. Fyson (1919–1923) proposed a section *Anisopetalae* and placed the species with unequal petals under this section. Zhang (1999) also adopted this section in his revisionary study. However, Ansari and Balakrishnan (1994, 2009) rejected this treatment, considering this character of little taxonomic value. We observed that all three types of petals are distributed throughout the genus and the plesiomorphic state for this character is equivocal (Fig. 8*b*, S9). Most white anther species are characterized by unequal petals (i.e. large median petal and minute lateral petals). Phillips (1997) observed that dimensions of floral parts change at maturity, especially in zygomorphic flowers. While studying the African *Eriocaulon* species, she observed that the long median petals elongate at anthesis in male flowers. During this study, we observed that the species characterized by unequal petals exhibit the largest median petals in the peripheral flowers, and so we presume that the unequal petals could be acting as a landing platform for insect pollinators. However, pollination studies on Indian *Eriocaulon* species are awaited.

Hairiness of involucral and floral bracts. Fyson (1919–1923) grouped all hairy species under section *Hirsutae*, however, Ansari and Balakrishnan (1994, 2009) considered this character of little taxonomic importance. Based on our observation of freshly collected material, we consider hairiness of involucral bracts and floral bracts as distinct characters as glabrous involucral bracts and hairy floral bracts appear in most



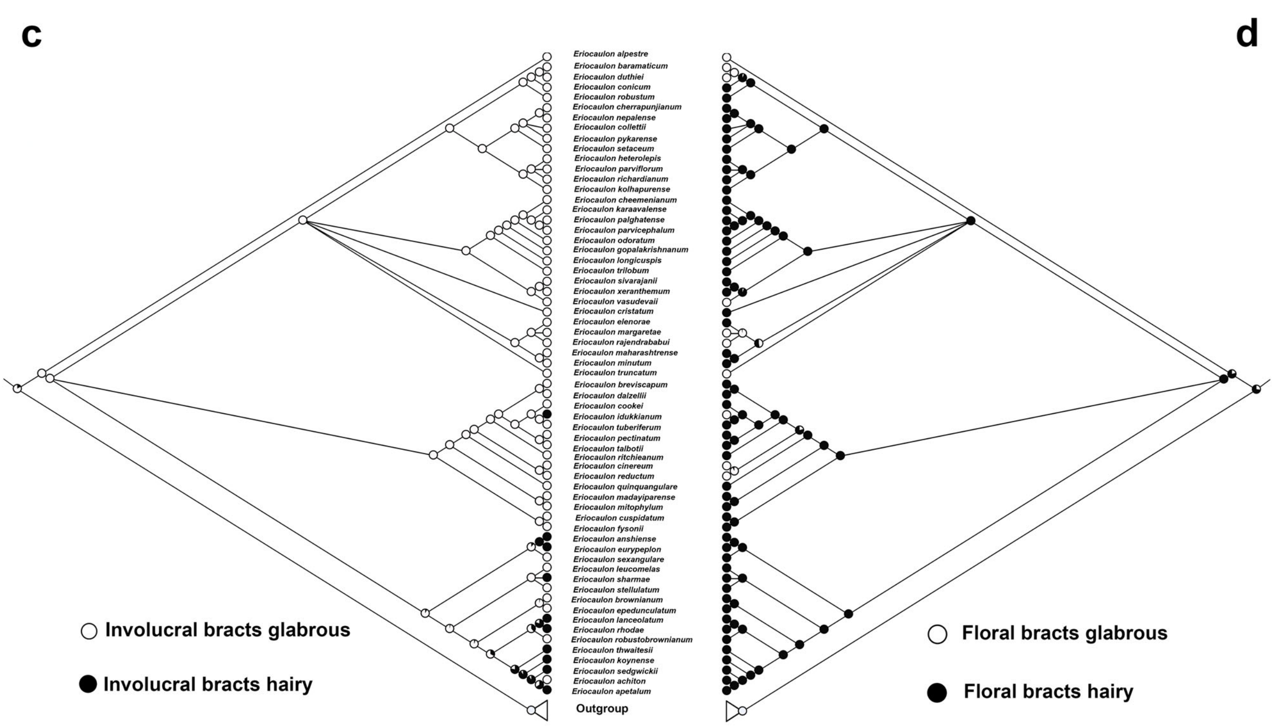


Fig. 8. Distribution of characters onto a Bayesian tree: a. Seed coat cell type, b. Size of male petals, c. Hairy or glabrous involucral bracts, d. Hairy or glabrous floral bracts.

of the Indian species (Fig. 8*c*, S10 and 8d, S11). The hairiness of sepals and petals of both male and female flowers exhibit a lot of intraspecific variation. Hence, these characters were not considered.

Glabrous involucral bracts are resolved as plesiomorphic while species with hairy involucres are restricted to clades II and III where the trait has evolved multiple times (Fig. 8*c*, S10). Variation in hairiness of involucral bracts was observed in *E. robustobrownianum*, *E. sedgwickii* and *E. brownianum* to some extent in one or two collected accessions. We observed floral bract hairiness as a constant character in Indian *Eriocaulon* species (Fig. 8*d*, S11). We assume hairiness of bracts, as well as receptacles, could be aiding in pollination and dispersal of seeds. We also observed that species such as *E. lanceolatum*, *E. thwaitesii* having hairy floral bracts and receptacles show pseudovivipary.

Stem. Most Indian *Eriocaulon* species are acaulescent herbs. However, the presence of a stem appears to be an apomorphic feature independently evolved in all the three clades (Fig. S12). Few of the species we collected (viz. *E. brownianum*, *E. cristatum* and *E. setaceum*) possess stems. Most of the annual Indian species occurring in seasonally wet areas are acaulescent herbs. *E. setaceum* is one of the widespread species of the genus which is completely aquatic (Leach, 2017; Phillips, 1997). The stem of *E. setaceum* is submerged below the surface of the water and grows to around 40 – 50 cm long. The stem is densely covered by filiform leaves along its length. Another caulescent Indian species is *E. brownianum* which is reported from the Western Ghats and North East Himalaya. Both *E. setaceum* and *E. brownianum* appear to be annual species. The third species, *E. cristatum*, is characterized by a pseudo-stem and is restricted to the North East region in India where it grows in seasonal pools and margins of wet bodies on the plateaus.

Rootstock. Rootstocks are the underground stem-like portion bearing roots (Ansari & Balakrishnan, 1994, 2009). Most of the Indian species lack a rootstock and this is plesiomorphic. A rootstock has evolved independently on several occasions and in two of the clades (Fig S13). Ansari and Balakrishnan (1994, 2009) considered the rootstock character of little taxonomic value. In contrast, many recently described Indian species have considered rootstock as a valid and distinguishing character (Manudev et al., 2017; Sunil et al., 2017). Our study suggests that the rootstock character is homoplastic.

Other morphological features

Eriocaulon alpestre Hook.f. & Thomson ex Körn. is the only Indian species having fused female sepals and is restricted to the Himalayan region in India. Many East Asian species, most of which were placed in section *Apoda* by Zhang (1999), viz. *E. buergerianum* Körn., *E. atroides* Satake etc. are also characterized by fused female sepals.

A few Australian and East Asian species such as *E. distichoides* Mangen, *E. escape* B.F.Hansen and *E. pseudoescape* Praj. & Chantar. lack peduncles (Leach, 2017; Zhang, 1999). In India, only *E. epedunculatum* Potdar, Anil Kumar bis, Otaghvari & Sonkar is devoid of peduncles and known only from type locality. The character was not considered for reconstruction due to a single evolutionary event (Uyeda et al., 2018).

It would be interesting to check evolution of these additional characters (fusion of female sepals and presence or absence of peduncles) after inclusion of species from other continents.

Taxonomic notes

The *Eriocaulon dianae* complex. While working on the Indian *Eriocaulon* species, Fyson (1919–1923) described *E. dianae* with seven new varieties, namely var. *typica*, *longibracteata*, *parviflora*, *richardiana*, *folia*, *triloboides* and *conica*. He, however, mentioned the species possesses a large number of intermediates having overlapping characters which makes separation of seven proposed varieties difficult. Ansari and Balakrishnan (1994, 2009) critically studied five of his varieties and raised them as four distinct species *E. conicum* (var. *conica*); *E. heterolepis* (var. *longibracteata*); *E. richardianum* (var. *richardiana*) and *E. parviflorum* (var. *parviflora* and var. *triloboides*). The other two varieties (var. *dianae* and var. *folia*) were not considered. Fyson (1919–1923) suggested that var. *folia* connects var. *typica* and var. *triloboides*. He also noted that the distribution of var. *typica* is from 'Salsette to S. Kanara' while that of var. *folia* on 'hills near Bombay'. The former was characterized by hemispherical, globose or ovoid heads, involucral bracts extending beyond the margin and the unequal sepals while the latter possesses globose heads with reflexed bracts and acicular leaves. During this study, *E. parviflorum* was collected from parts of Goa, Karnataka and Maharashtra and extreme variations in the habit, size and shape of head, and involucral bracts were observed. Also, multiple accessions of *E. heterolepis*, *E. richardianum* and *E. conicum* were collected and critically examined (Supplementary Appendix 1). In the present study, *Eriocaulon heterolepis*, *E. parviflorum* and *E. richardianum* (all sect. III)

were resolved in a well-supported trichotomy (BP = 100, PP = 1) in clade I (Fig. 6). *E. conicum* which is morphologically distant from these three species was placed in another subclade of the same clade. It possesses distinct seed characters, bracts as well as male and female sepals and so it was placed in section VII by Ansari and Balakrishnan (1994, 2009). Though molecularly not distinct, we believe these three are distinct species evidenced from differences in habit, rootstock (in *E. richardianum*) and involucral bracts. A scrutiny of multiple accessions also revealed intermediate forms between *E. parviflorum* and *E. heterolepis* which could be the varieties *dianae* and *folia* described by Fyson. Population studies are required to resolve the status of the two varieties proposed by Fyson as well as the whole 'dianae' complex.

***Eriocaulon cinereum–Eriocaulon redactum* complex.** Ruhland (1903) established *E. redactum* and distinguished it from *E. sieboldianum* (=*E. cinereum*) based on reduced female sepals. Considering the morphological plasticity exhibited by the genus, Fyson (1919–1923) treated it as conspecific to *E. sieboldianum*. Khanna and Kumar (2000) compared 12 specimens for characters of the head (colour), involucral bracts (colour), floral bracts (shape), spathe of male flowers (shape and hairiness) and seeds (colour and size). They found several intermediate characters and hence treated the two species as conspecific. Later, Ansari and Balakrishnan (1994, 2009) treated these two as separate entities and claimed that Khanna and Kumar (2000) had ignored the female sepals. During this study, several accessions of this complex were collected and examined (Supplementary Appendix 1). They exhibited uniformity in characters that allowed two taxa to be differentiated with *E. cinereum* characterized by a columnar receptacle and one or two persistent female sepals while *E. redactum* possesses a convex receptacle and female sepals reduced in the form of hairs (Fig. 9 I & II). Population studies using four different molecular loci (*rbcL*, *psbA*-*trnH*, ITS and *trnL*-*F*; unpublished data) also supported the recognition of these two species as separate entities in agreement with the study of Ansari and Balakrishnan (1994, 2009). The *psbA*-*trnH* locus exhibited 29 variable sites and 12 parsimony informative sites while *trnL–F* region exhibited 18 variable and 16 parsimony informative sites.

Other species complexes

After a critical scrutiny of freshly collected specimens, type materials, multiple voucher specimens deposited in various herbaria, relevant literature (Ansari & Balakrishnan, 1994, 2009; Anto & Reshma, 2017; Biju et al., 2012; 2018; Chandore et al., 2019; Darshetkar et al., 2017; 2019; Francis et al., 2020; Khanna and Kumar, 2019; Manudev et al., 2017; Nampy et al., 2011; Naveen Kumar et al., 2017; Paithane et al., 2017; Rashmi & Krishnakumar, 2014; Shimpale et al., 2009; Shimpale & Yadav, 2010; Sunil et al., 2013; 2015; 2017; Sunil & Kumar, 2015; Swapna et al., 2012; Vivek et al., 2010) and sequence data from cpDNA and nrDNA, as discussed above, the following taxonomic changes are proposed.

1. *Eriocaulon gulnarparianum* Paithane et al. versus *E. xeranthemum* Mart. In the protologue, *Eriocaulon gulnarparianum* was compared with *E. xeranthemum* and *E. devendranii* Vijaya Sankar et al. for several quantitative and morphologically plastic characters (Paithane et al., 2017). The species was differentiated by leaves 0.8 mm (this measurement was erroneous) to 1.5 cm long whereas in *E. xeranthemum* they are up to 4×0.3 cm and in *E. devendranii*, up to 1 cm long. The other characters considered for species discrimination were: involucral bracts 2–3.5 cm in length (vs 3 cm in *E. xeranthemum* and 3.5 cm in *E. devendranii*); oblong oblanceolate floral bracts (vs. truncate in *E. xeranthemum* and oblanceolate in *E. devendranii*); obtuse three lobed male sepals (two connate, one free which is slightly joined at base) (vs. three lobed obtuse in *E. devendranii* and truncate in *E. xeranthemum*); spathulate one or two notched female petals (vs. spathulate petals in *E. xeranthemum* and *E. devendranii*) and $1-2$ setiform seed appendages (vs. $2-4$ in *E. devendranii* and *E. xeranthemum*). The number of seed appendages is a variable character for the genus as is well documented (Ansari & Balakrishnan 1994, 2009). Paithane et al. (2017) stated that the species is characterized by the presence of $1-2$ appendages on the seed surface but the scanning electron microscopy (SEM) images accompanying the original description do not exhibit the presence of appendages. As the species is described based largely on quantitative and plastic characters, we suggest *Eriocaulon gulnarparianum* is treated conspecific with *E. xeranthemum*.

***Eriocaulon xeranthemum* Mart.** Pl. Asiat. Rar. (Wallich). 3(10): 29 (1832).

= *Eriocaulon gulnarparianum* Paithane, Bhuktar A.S., Kashetti R.P. & Patil S.B., Int. J. Adv. Res. 5(10), 859 (2017), ***syn. nov.***

2. *Eriocaulon govindiana* Sunil & Ratheesh versus *E. nepalense* Prescott ex Bong. *Eriocaulon govindiana* is morphologically similar to *E. nepalense* and characters used by Sunil et al. (2017) to distinguish them (such as

hairiness of sepals and petals, the shape of the apex, number of sepals) show variation within species. Sunil et al. (2017) distinguished *E. govindiana* from *E. nepalense* by its leaves up to 3 – 7 cm long, with scabrid margin and rounded apices (vs. leaves up to 10 cm long, smooth margin and subacuminate to obtuse apex); male sepals that are 1.5 – 1.7 cm long, 3-lobed, glabrous and with acute to subacuminate apex (vs. 1.5 cm long, 3 lobed hairy male sepals with obtuse apex); one or three hairy male petals (vs. three glabrous male petals); two or three unequal female sepals (vs. three subsimilar female sepals); non-pilose sparsely hairy female petals (vs. pilose, not hairy female petals) and 1 – 3 setiform seed appendages (vs. 1 – 4 setiform seed appendages). The illustration provided with the protologue exhibits one male petal, however, the photograph provided shows three male petals. The only striking and valid diagnostic feature claimed by the authors is the presence of a rootstock which is neither shown in illustration nor in the photoplate. Ansari and Balakrishnan (1994, 2009) noted that the rootstock is of little taxonomic importance in the genus. Also, there is confusion regarding what part should be considered a rootstock in *Eriocaulon*. Based on these observations, we suggest *Eriocaulon govindiana* is treated as conspecific with *E. nepalense* Prescott ex Bong.

Eriocaulon nepalense **Prescott ex Bong**. Mém. Acad. Imp. Sci. St. Pétersbourg, Sér. 6, Sci. Math. 1: 610 (1831).

= *Eriocaulon govindiana* Sunil & Ratheesh, Taiwania 62(4): 387 (2017), ***syn. nov.***

3. *Eriocaulon pradeepii* Anto & Reshma versus *E. palghatense* R. Ansari & N.P.Balakr. *Eriocaulon pradeepii* is morphologically allied to *E. palghatense* R.Ansari & N.P. Balakr. in its small stature, minute inflorescence, glandular petals in the male and female flowers, and ribbon-shaped seed appendages. However, Anto and Reshma (2017) overlooked these similarities and did not compare these two species when *E. pradeepii* was described. Rather, they compared *E. pradeepii* with *E. quinquangulare* and *E. cristatum* which are apparently not the close allies of this species based on morphology. *Eriocaulon pradeepii* appears to differ from *E. palghatense* by the appearance of five narrow lines on the seed but this would appear to be intraspecific variation and we suggest that these two species should be treated as conspecific.

Eriocaulon palghatense R.Ansari & N.P.Balakr. Fam. Eriocaulac. in India 111 (1994).

=*Eriocaulon pradeepii* Anto & Reshma, Taiwania 62(4): 371 (2017), ***syn. nov.***

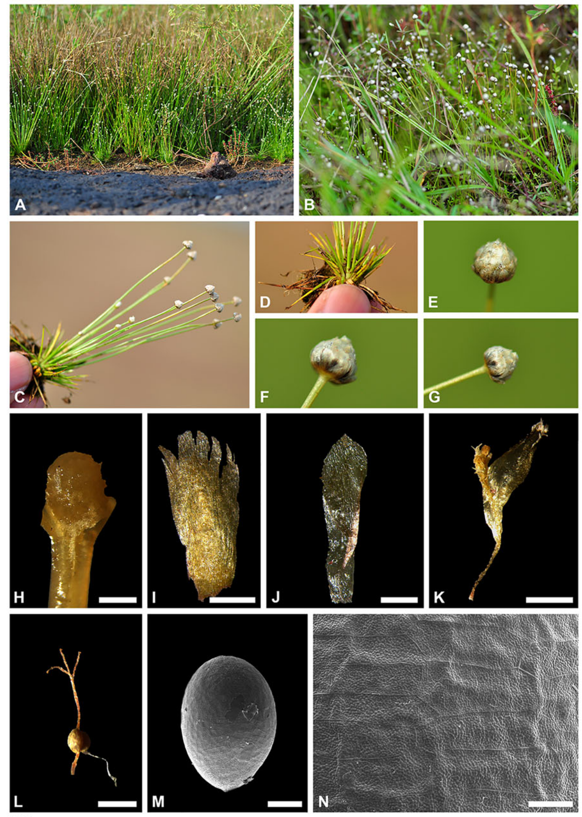
4. *Eriocaulon idukkianum* Manudev et al. versus *E. tuberiferum* A.R. Kulk. & Desai. A critical study of morphology along with molecular data suggests *E. idukkianum* should be considered conspecific with *E. tuberiferum*. Manudev et al. (2017) compared *E. idukkianum* with *E. tuberiferum* for characters such as presence of rootstock (vs. absence of rootstock); single glabrous tuber (vs. 2 – 15 hairy tubers); pilose receptacles (vs. glabrous receptacles); free male sepals (vs. fused male sepals) and absence of seed appendages (vs. ribbon-shaped seed appendages). However, some of these characters are problematic. First, during this study, several accessions of *E. tuberiferum* were collected, many of which exhibit the presence of a single tuber. Second, the image of the habit provided in the protologue exhibits roots but no rootstock. The only notable difference given by the authors is the presence or absence of seed appendages for which the protologue authors provide neither SEM images nor the illustrations. During the present study, SEM of seeds revealed appendages in *E. idukkianum* similar to those of *E. tuberiferum*. Also, no molecular divergence was observed between two species (Fig. 6). Based on these observations, we suggest synonymization of *Eriocaulon idukkianum* with *E. tuberiferum.*

Eriocaulon tuberiferum A.R.Kulk. & Desai J. Bombay Nat. Hist. Soc., 71(1): 81. 1974.

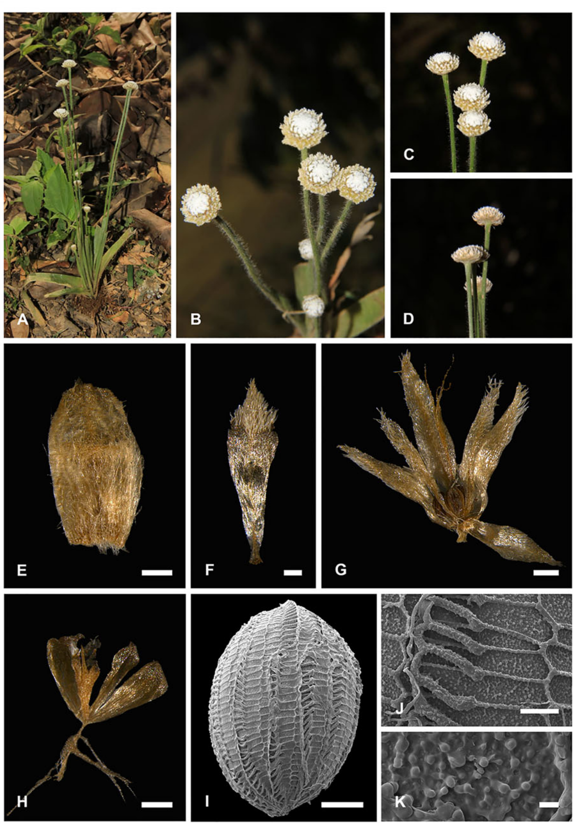
=*Eriocaulon idukkianum* Manudev, Robi & Nampy, Phytotaxa 324(3): 289 (2017), ***syn. nov.***

5. *Eriocaulon baramaticum* Shimpale et al. versus *E. duthiei* Hook.f. The scrutiny of freshly collected and multiple herbarium specimens revealed that *E. baramaticum* is allied with *E. duthiei.* Indeed, it only differs from *E. duthiei* in the absence of seed appendages. In the protologue, *E. baramaticum* was compared with *E. rajendrababui* R. Ansari & N.P. Balakr. (Shimpale et al., 2009), a species belonging to a distantly placed section XI (Ansari & Balakrishnan 2009), from which it differs in involucral bracts, number of sepals and seed characters. However, in this study, molecular data placed *E. baramaticum* sister to *E. duthiei* (BP = 100, PP = 1). Our critical scrutiny of the morphological characters suggests *E. baramaticum* is conspecific with *E. duthiei.* Both possess eglandular petals, glabrous floral parts, shape of involucral and floral bracts. They only differ in number of sepals (2 or 3 in the former and 2 in the latter) and seed appendages (absent in the former while setiform seed appendages in the latter). During the present study, multiple specimens of *E. duthiei* were studied and variation in the number of sepals was observed. In some, only two sepals were present while in some heads, a few flowers had two sepals and some had three sepals (Fig. S4). The two species, *E. duthiei* and *E. baramaticum* differ only in the character of seed

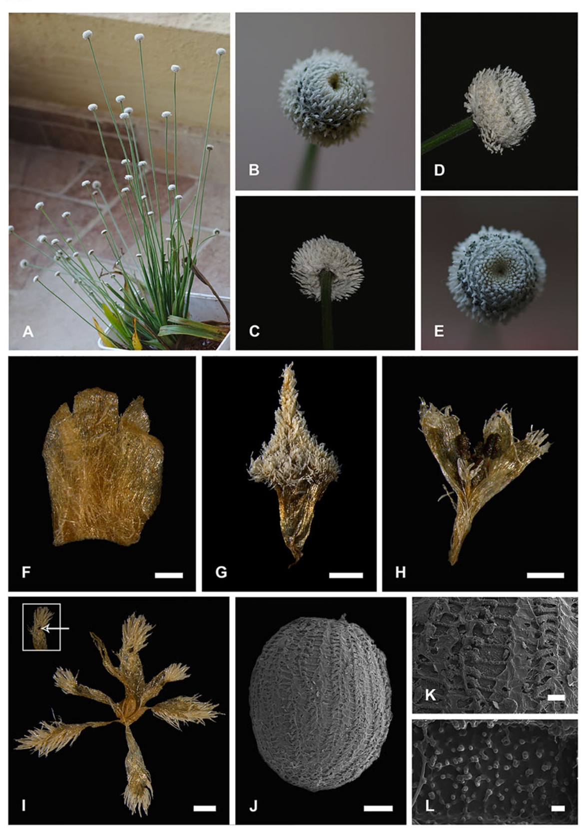
I



II



III



IV

appendages. In addition, both the species exhibited no molecular divergence in sequences of *rbcL*, *psbA-trnH*, *trnL–F* and ITS markers (Darshetkar, 2021).

Eriocaulon duthiei Hook.f., Fl. Brit. India [J. D. Hooker] 6(19): 578 (1893).

= *Eriocaulon baramaticum* Shimpale, Bhagat, R.B.Deshmukh & S.R.Yadav, Rheedea 19(1–2): 47 (–49; fig. 1) (2009), ***syn. nov.***

6. *Eriocaulon maharashtrense* Punekar & Lakshmin. versus *E. minutum* Hook.f. *Eriocaulon maharashtrense* is known only from the type locality. According to Punekar et al. (2003), it differs from its close ally *E. minutum* in having small leaves and sheaths up to 7 mm (vs. leaves up to 3.5 cm long and sheaths up to 1.5 cm long); a conical (vs. cylindric) receptacle; non-keeled (vs. keeled) female sepals and transversely elongated (vs. isodiametric) seed cells. However, the characters used are mostly quantitative and overlapping. During this study, several accessions of *E. minutum* were collected and variations in the characters were observed. We found it difficult to distinguish between conical and cylindrical receptacles due to their minute size and variation observed in multiple accessions. We also observed variation in the seed coat cell shape of *E. minutum* which varied from transversely elongated to isodiametric in different accessions collected from the type locality (Fig. S4). Therefore, these two species should be considered as conspecific. Moreover, we observed no divergence between these species in *trnL–F*, which supports our morphological observation (Fig. 6).

Eriocaulon minutum Hook.f., Fl. Brit. India [J. D. Hooker] 6(19): 579 (1893).

= *Eriocaulon maharashtrense* Punekar, et al., Rheedea 13(1 – 2): 24 (fig. 4) (2004), ***syn. nov.***

7. *Eriocaulon apetalum* Punekar et al. versus *E. achiton* Körn. *Eriocaulon apetalum,* known only from type locality, was distinguished from *E. achiton* in having convex receptacle (vs. discoid receptacle); sparsely hairy involucral bracts (vs. glabrous involucral bracts); female sepals canaliculate with subacute to acute apices (vs. female sepals linear with subacute apices) and seeds without appendages (vs. seeds with 1 – 2 setiform seed appendages). The characters considered for discriminating the species exhibit extreme variations except for the seed character. During the present study, several accessions of *E. achiton* were collected from the Western Ghats, and Meghalaya. *E. apetalum* was also collected from its type locality. Both species were critically studied for their morphology and molecular divergence. No molecular divergence was observed between the two species in both the studied loci. As we have observed and included single accession of *E. apetalum* in this study, inclusion of more accessions and multiple molecular markers would help in resolving the status of the species. Nonetheless, we have treated the species here as distinct entities.

8. *Eriocaulon anshiense* Punekar et al. versus *E. eurypeplon* Körn. *Eriocaulon anshiense* differs from its closest allied species *E. eurypeplon* in having spinulate heads (vs. globose heads); acuminate involucral bracts (vs. obtuse or subacute involucral bracts); floral bracts caudate at apex (vs. acuminate floral bracts); acuminate female sepals, exceeding the floral bracts, 2–2.2 mm long (vs. acute or obtuse female sepals, 1.2–1.5 mm long) and beaked seeds (vs. non-beaked seeds). During this study, we observed extreme variations in *E. eurypeplon* in the above-mentioned characters. The ITS dataset did not show any variation between these species; however, some variation in the *trnL–F* locus could be observed. Adding data from multiple accessions and multiple loci of both species will help to understand the status of this species.

9. *Eriocaulon vamanae* Dani & Nampy versus *E. thwaitesii* Körn. *Eriocaulon vamanae* should be treated as conspecific with *E. thwaitesii* as there are no clear morphological differences between these two species. Francis et al. (2020) have compared *E. vamanae* with *E. nepalense* var. *luzulaefolium*, *E. duthiei* and *E. thwaitesii* using morphological characters, most of which were quantitative and overlapping (e.g. sepals in female flower 1.6–2 mm in *E. vamanae* and 1.75–2 mm in *E. thwaitesii*). Other characters used include receptacles obovoid and pilose (vs. convex villous receptacles in *E. thwaitesii*); male petals hairy towards apex (vs. sparsely hairy towards apex). It is difficult to distinguish between hairy and sparsely hairy petals as a lot of variation was observed in the degree of hairiness of *E. thwaitesii*, during this study. Similarly, considering minute size of

Fig. 9. I. *Eriocaulon cinereum* (A. Habitat, B. Habit, C. Leaves, D. & E. Heads, F. Receptacle, G. Involucral bract, H. Floral bract, I. Male flower, J. Female flower, K. Photomicrograph of seed, L. Seed appendages); II. *Eriocaulon reductum* (A. & B. Habitat, C. Habit, D. Leaves, E. to G. Heads, H. Receptacle, I. Involucral bract, J. Floral bract, K. Male flower, L. Female flower, M. Photomicrograph of seed, N. Seed appendages); III. *Eriocaulon rhodae* (A. Habit and Habitat, B. to D. Heads, E. Involucral bract, F. Floral bract, G. Female flower, H. Male flower, I. Photomicrograph of seed, J. Seed appendages, K. Seed surface); IV. *Eriocaulon robustobrownianum* (A. Habit, B. to E. Heads, F. Involucral bract, G. Floral bract, H. Male flower, I. Female flower, J. Photomicrograph of seed, K. Seed appendages, L. Seed surface).

receptacles, it is difficult to discriminate between obovoid and convex receptacles. *Eriocaulon thwaitesii* is widely distributed in Southern Western Ghats and occurs frequently in Kerala. During our study, we observed extreme variations in the habit and in the male and female flowers of *E. thwaitesii*, mostly in quantitative traits and in the degree of hairiness. Indeed, the intraspecific variations which we observed in the collected accessions of *E. thwaitesii* includes characters used to distinguish *E. vamanae*. A critical study of the type specimens along with multiple accessions using molecular data would be required to confirm the status of *E. vamanae*.

Reinstatement of *Eriocaulon* rhodae

Species having solitary seed appendages and acuminate or subacuminate floral bracts, viz. *Eriocaulon lanceolatum*, *E. rhodae* and *E. robustobrownianum* were resolved as a clade (BP = 95, PP = 1). *Eriocaulon rhodae* and *E. lanceolatum* are endemic to India while *E. robustobrownianum* is distributed also in South East Asia.

Govaerts (2001) accepted the name *E. rhodae* while preparing World Checklist of family Eriocaulaceae but later accepted Zhang's treatment for WCSP (2019), presumably the most updated online database, following which all subsequent taxonomic databases (such as Roskov et al. 2019; World Flora online, 2019; POWO, 2019) consider *E. rhodae* as a synonym of *E. robustobrownianum*. Our scrutiny of multiple specimens (Supplementary Appendix 1) revealed *E. rhodae* to be clearly different from *E. robustobrownianum* in having oblong leaves (vs. oblong-ensiform leaves), acuminate floral bracts (vs. acicular floral bracts), eglandular female petals (vs. glandular female petals), acute, oblong-ellipsoid seeds with foveate seed surface (vs. obtuse, oblong-ovoid seeds with foveaolate seed surface) (Fig. 9 III & IV). The molecular data also confirmed them as two distinct identities (Fig. 6). Hence, *E. rhodae* and *E. robustobrownianum* should be treated as separate entities.

Key for identification of Indian *Eriocaulon* species

1a. Female sepals connate into a spathe	1. *E. alpestre*
1b. Female sepals free	2
2a. Peduncles absent	2. *E. epedunculatum*
2b. Peduncles present	3
3a. Tubers present	3. *E. tuberiferum*
3b. Tubers absent	4
4a. Leaves papillose	4. *E. gopalakrishnanum*
4b. Leaves smooth	5
5a. Anthers 3	5. *E. vasudevanii*
5b. Anthers 4 or 6	6
6a. Female petals suppressed or absent	7
6b. Female petals present	8
7a. Anthers black	9
7b. Anthers white	10
8a. Leaves purple or turning purplish on drying	11
8b. Leaves never turning purplish	12
9a. Male sepals connate into a spathe	13
9b. Male sepals free	14
10a. Receptacle columnar, female sepals persistent, 1 or 2	6. *E. cinereum*
10b. Receptacle convex, female sepals reduced in the form of hairs	7. *E. redactum*
11a. Male and female sepals glabrous, involucral bracts elliptic, longer than floral bracts	12. *E. martianum*
11b. Male and female sepals hairy, involucral bracts obovate, shorter than floral bracts	15
12a. Leaf apex cuspidate	16
12b. Leaf apex either acute, apiculate, acuminate or obtuse	18
13a. Floral bracts lanceolate, acuminate	8. *E. echinulatum*
13b. Floral bracts oblong-elliptic, cuspidate	9. *E. chandrae*
14a. Seed appendages present (1–2), arising from transverse radial walls	10. *E. achiton*
14b. Seed appendages absent	11. *E. apetalum*
15a. Involucral bracts acute, male petals unequal	13. *E. mirzapurense*
15b. Involucral bracts obtuse, male petals subequal	14. *E. quinquangulare*

(*continued*)

16a. Involucral bracts distinctly exceeding the head, seed appendages dilated at the apex	15. *E. fysonii*
16b. Involucral bracts not exceeding the head, seed appendages truncate at the apex	17
17a. Rootstock absent	16. *E. cuspidatum*
17b. Rootstock present	17. *E. rayatianum*
18a. Anthers white	19
18b. Anthers black	32
19a. Seed surface with appendages	20
19b. Seed surface without appendages	21
20a. Seed appendages solitary setiform arising from transverse radial walls	18. *E. albotetrandra*
20b. Seed appendages in the form of ribbon-like bands arising from transverse radial walls	19. *E. periyarense*
21a. Seed coat cells isodiametric	20. *E. manoharanii*
21b. Seed coat cells transversely elongated	22
22a. Leaves filiform, abruptly broadening at base	23
22b. Leaves linear or linear ensiform, gradually broadening at base	24
23a. Spathe of male flowers with truncate lobes, female petals eglandular	21. *E. barbeyanum*
23b. Spathe of male flowers obtuse or acute lobes, female petals glandular	25
24a. Leaves up to 30 cm long, male petals unequal, middle petal longer than the lateral ones	22. *E. breviscapum*
24b. Leaves not more than 15 cm long, male petals subequal	26
25a. Filaments of stamens elongated and twisted	23. *E. miserum*
25b. Filaments of stamens short and straight	27
26a. Female sepals narrowly linear	28
26b. Female sepals broadly oblong or oblanceolate	29
27a. Plants entirely submerged, leaves as long as peduncles, heads hemispherical	24. *E. fluviatile*
27b. Plants emergent, leaves much shorter than peduncles, heads globose	25. *E. mitophylum*
28a. Receptacles globose, floral bracts acuminate, male petals hoary, seeds ovoid	26. *E. panagudianum*
28b. Receptacles concave, floral bracts acute, male petals glabrous, seeds oblong-ellipsoid	27. *E. ratnagiricus*
29a. Involucral bracts acute, receptacles flat or convex	28. *E. leucomelas*
29b. Involucral bracts obtuse, receptacles cylindric or conical	30
30a. Peduncles 1 or 2	29. *E. cookei*
30b. Peduncles many	31
31a. Male sepal lobes acute	30. *E. talbotii*
31b. Male sepal lobes obtuse	31. *E. ritchieanum*
32a. Seed surface furnished with appendages or ridges	33
32b. Seed surface without appendages or ridges	37
33a. Seed coat cells isodiametric	34
33b. Seed coat cells transversely elongated	38
34a. Stems present, leaves cauline, setiform appendages arising from angles of the walls	35
34b. Stems absent, leaves rosulate, setiform appendages arising from all radial walls	36
35a. Rootstock up to 5 cm long, stem branched, male sepals fused, male petals unequal, appendages setiform	32. *E. nairii*
35b. Rootstock absent, stem tufted, male sepals free, male petals subequal, appendages setiform and ribbon shaped	33. *E. biappendiculatum*
36a. Rootstock present, male and female petals unequal, appendages setiform truncate at apex	34. *E. robustum*
36b. Rootstock absent, male and female petals subequal, appendages setiform dilated at apex	35. *E. collettii*
37a. Vertical radial walls of seed coat cells thickened	36. *E. cristatum*
37b. Vertical radial walls of seed coat cells not thickened	88

(*continued*)

38a. Seed appendages ribbon like or rectangular or represented by ridges on the surface of seeds	39
38b. Seed appendages setiform	50
39a. Male flowers with three sepals	40
39b. Male flowers with two sepals	41
40a. Male sepals free	37. *E. longicuspe*
40b. Male sepals connate into a spathe	42
41a. Cells of seed coat vertically elongated	38. *E. truncatum*
41b. Cells of seed coat laterally elongated	47
42a. Seed appendages arising from both transverse and vertical radial walls	39. *E. cherrapunjianum*
42b. Seed appendages arising from the transverse radial walls alone	43
43a. Seed appendages in the form of 2 or 2–4 rectangular bands	44
43b. Seed appendages solitary, ribbon like bands conforming to the length of cells	45
44a. Male petals glandular and female petals eglandular	40. *E. bastarense*
44b. Male petals eglandular and female petals glandular	41. *E. parvicephalum*
45a. Involucral bracts oblong-oblanceolate	42. *E. conicum*
45b. Involucral bracts obovate	46
46a. Heads globose, floral bracts acute or obtuse, male petals subequal	43. *E. palghatense*
46b. Heads hemispherical, floral bracts acuminate, male petals unequal	44. *E. karaavalense*
47a. Male sepals connate into a spathe	45. *E. edwardii*
47b. Male sepals free	48
48a. Involucral bracts much longer than floral bracts, erect or spreading	46. *E. raipurense*
48b. Involucral bracts equal to or shorter than floral bracts, reflexed	49
49a. Female petals linear, eglandular, floral bracts glabrous	47. *E. hamiltonianum*
49b. Female petals spathulate, glandular, floral bracts pilose	48. *E. ramnadense*
50a. Male sepals free	51
50b. Male sepals connate laterally at least at the base to form a closed or open spathe	52
51a. Involucral and floral bracts dimorphic	49. *E. koynense*
51a. Involucral and floral bracts monomorphic	53
52a. Spathe of male flowers closed and tubular	57
52b. Spathe of male flowers split on the abaxial side, open	58
53a. Female sepals without a keel at back	54
53b. Female sepals broadly keeled at back	55
54a. Involucral bracts, floral bracts, sepals and petals densely hairy at apex, glandular male and female petals	50. *E. sedgwickii*
54b. Involucral bracts, floral bracts, sepals and petals glabrous, eglandular male and female petals	51. *E. duthiei*
55a. Female sepals acuminate, exceeding the floral bracts	52. *E. anshiense*
55b. Female sepals obtuse or acute, not exceeding the floral bracts	56
56a. Female sepals entire, female petals equal	53. *E. eurypeplon*
56b. Female sepals irregularly toothed towards apex, female petals subequal	54. *E. madayiparense*
57a. Flowers dimerous, female petals equalling the sepals	55. *E. longifolium*
57b. Flowers trimerous, female petals shorter than the sepals	59
58a. Stem more than 10 cm long, leaves cauline, filiform	56. *E. setaceum*
58b. Stem absent or less than 5 cm long, leaves caespitose, not filiform	60
59a. Keels of female sepals lyrate, female petals longer than pistil, seeds oblong-ellipsoid	57. *E. peninsulare*
59b. Keels of female sepals almost entire, female petals shorter than pistil, seeds ovoid or globose	58. *E. sexangulare*
60a. Female petals coriaceous, broad, with a distinct claw	61
60b. Female petals hyaline, narrow, not clawed	62
61a. Male petals with a linear apicule, peduncles rigid	59. *E. rhodae*

(*continued*)

61b. Male petals without apicule, peduncles not rigid	60. *E. helferi*
62a. Spathe of male flowers 2-lobed, with or without a minute protuberance in between	63
62b. Spathe of male flowers 3-lobed	64
63a. Leaves linear, ~2 mm broad at base, peduncles many, involucral bracts oblong	61. *E. thwaitesii*
63b. Leaves oblong-ensiform, ~25 mm broad at base, peduncles 1 or 2, involucral bracts obovate-orbicular	62. *E. ensiforme*
64a. Female sepals equal or subequal	65
64b. Female sepals unequal, the odd sepal distinctly narrower than the lateral ones	66
65a. Seed appendages always solitary from the transverse radial walls, never from the vertical walls	67
65b. Seed appendages few – many (rarely solitary in few cells) from the transverse radial walls, occasionally from the vertical walls	68
66a. Odd middle female sepal distinctly larger than the lateral ones	63. *E. wayanadense*
66b. Odd middle female sepal smaller than the lateral ones or absent	82
67a. Leaves lanceolate	69
67b. Leaves narrowly oblong or linear	70
68a. Seed appendages arising from the middle of transverse radial walls of each cell so that they appear in vertical rows on the seed surface	71
68b. Seed appendages spread uniformly on the transverse radial walls so that they appear in close transverse rings on the seed surface	72
69a. Male petals obovate, involucral bracts acuminate, appendages of seed coat curved and connate with the adjacent ones of the same row of seed coat cells forming a looped longitudinal line	64. *E. balakrishnanii*
69b. Male petals linear, involucral bracts obtuse, setiform seed appendages, not connate	65. *E. lanceolatum*
70a. Seed appendages curved and connate forming a looped longitudinal line, female sepals oblanceolate	66. *E. robustobrownianum*
70b. Seed appendages free from each other, female sepals ovate to obovate	67. *E. brownianum*
71a. Leaves linear-lanceolate, peduncles many	68. *E. nepalense*
71b. Leaves oblong, peduncles 1 to 3	69. *E. hookerianum*
72a. Pedicels of flowers woolly, involucral bracts pilose, leaves more than 1 cm wide	70. *E. sharmae*
72b. Pedicels of flowers glabrous, involucral bracts glabrous, leaves up to 1 cm wide	73
73a. Floral bracts lanceolate, much longer than involucral bracts, stellately spreading	71. *E. stellulatum*
73b. Floral bracts cuneate or oblanceolate, not distinctly larger than the involucral bracts, not stellately spreading	74
74a. Female petals eglandular	72. *E. oryzetorum*
74b. Female petals glandular	75
75a. Male petals unequal, the middle one larger than the others	76
75b. Male petals equal	77
76a. Rootstock up to 6 cm long, involucral bracts acuminate	73. *E. karnatakense*
76b. Rootstock absent, involucral bracts obtuse	78
77a. Rootstock absent	74. *E. trilobum*
77b. Rootstock present	80
78a. Flowers sessile, male odd petal ovate-elliptic, floral bracts obtuse	75. *E. cheemenianum*
78b. Flowers pedicelled, male odd petal linear-oblong, floral bracts acuminate	79
79a. Female sepals 3, setiform seed appendages 4 – 13, truncate at apex	76. *E. odoratum*

(*continued*)

79b. Female sepals 2, setiform seed appendages 2 – 5, dilated at apex 77. *E. kolhapurense*	
80a. Male petals glandular	78. *E. ansarii*
80b. Male petals eglandular	81
81a. Female sepals dimorphic, seed appendages 1 – 3 arising from transverse radial walls	79. *E. malabaricum*
81b. Female sepals monomorphic, seed appendages 9 – 11 arising from transverse radial walls, 2 from vertical radial walls	80. *E. pykarense*
82a. Involucral bracts obtuse or subacute	83
82b. Involucral bracts acuminate or cuspidate	84
83a. Involucral bracts exceeding the head, more than twice the length of floral bracts	81. *E. heterolepis*
83b. Involucral bracts not exceeding the head, less than twice the length of floral bracts	85
84a. Seed appendages exceeding the breadth of seed coat cells	82. *E. sivarajanii*
84b. Seed appendages not exceeding the breadth of seed coat cells	86
85a. Rootstock present, about 1.5 cm long	83. *E. richardianum*
85b. Rootstock absent	87
86a. Involucral bracts ovate-oblong or oblanceolate, floral bracts truncate at apex, lobes of male sepals truncate	84. *E. xeranthemum*
86b. Involucral bracts ovate-oblong, floral bracts obtuse at apex, lobes of male sepals obtuse	85. *E. devendranii*
87a. Female petals glabrous	86. *E. santapaui*
87b. Female petals hairy	87. *E. parviflorum*
88a. Seed coat cells isodiametric	89
88b. Seed coat cells transversely elongated	90
89a. Female sepals 2	88. *E. minutum*
89b. Female sepals 3	91
90a. Seed coat cells vertically elongated	89. *E. vandaanamense*
90b. Seed coat cells transversely elongated	92
91a. Female petals almost equalling the length of sepals, keel in odd female sepal saccate	90. *E. elenorae*
91b. Female petals distinctly shorter than the sepals, keel in the odd female sepal convex	93
92a. Male petals distinctly unequal	91. *E. kanarense*
92b. Male petals equal or subequal	94
93a. Spathe of male flowers pilose towards apex, floral bracts sparsely pilose	92. *E. meeboldii*
93b. Spathe of male flowers glabrous, floral bracts glabrous	93. *E. margaretae*
94a. Male and female flowers glabrous, female petals eglandular	94. *E. rajendrababui*
94b. Male and female flowers hairy, female petals glandular	95
95a. Female flowers sessile, floral bracts truncate	95. *E. belgaumensis*
95b. Female flowers pedicelled, floral bracts acuminate	96. *E. pectinatum*

Species Excluded: *E. barba-caprae*, *E. gregatum*, *E. glaucum*, *E. pumilo* and *E. rouxianum* were excluded following Ansari and Balakrishnan (1994, 2009). Also, these species could not be collected during our field surveys. Further, no mature flowers were encountered in any herbaria for detailed study. *E. vamanae* is closely allied to *E. thwaitesii*, however, it could not be placed in the key due to lack of striking morphological features from its closest ally (as discussed above). *E. bolei*, known only from the type locality was not mentioned in the treatment proposed by Ansari and Balakrishnan (1994, 2009). The species could not be collected after several attempts. As the floral characters are imperfectly known, the species could not be placed in the key.

Synonymized and excluded: *E. gulnarparianum*, *E. pradeepii*, *E. govindiana*, *E. maharashtrense*, *E. baramaticum*, *E. idukkianum.*

Conclusion

The present phylogenetic study is the first effort to understand the relationships between *Eriocaulon* species from India with more than 60% species representation

covering all sections proposed by earlier workers. This is also the first comprehensive study where extensive field collections were made for the genus after Fyson's century-old work. The explorations yielded the rediscovery of *E. collettii* after four decades. Moreover, the study investigates the morphological evolution within the genus. Earlier studies mostly focused on the evolution of *Paepalanthoideae* members and included sparse sampling from *Eriocauloideae*. In this study, more emphasis was given on comparing the results of molecular analyses with the sectional treatment proposed by the earlier workers. The results exhibited little congruence with the sectional treatment proposed by Ansari and Balakrishnan (1994, 2009) and provided insights for the selection of characters important for delimitations. In addition, the reliability of morphological characters considered of prime importance in species delimitation was tested. Considering the extent of homoplasy, it appears difficult to recognize apomorphies to delimit groups. However, anther colour, presence/absence of peduncles, and fusion of female sepals could play important roles in classifying *Eriocaulon* species. The study calls into question the circumscription of several *Eriocaulon* species such as *E. baramaticum* (vs *E. duthiei*), *E. idukkianum* (vs. *tuberiferum*), *E. apetalum* (vs *E. achiton*) that were based on ambiguous taxonomic characters. Critical scrutiny of the freshly collected plant materials showed intraspecific variation in critical characters previously used to delimit a number of species (Supplementary Appendix 1).

The identification of white anther species is challenging because of the lack of characters used for delimitation of the Indian *Eriocaulon* species. All white anther species studied during the present work lack characteristic seed appendages, and most of them have unequal petals which is difficult to confirm in the preserved specimens. In this study, all white anther species except *E. leucomales* were resolved under one clade.

A number of recently described *Eriocaulon* species (discussed above) are creating a lot of confusion among taxonomists. Minor variations within species have been looked upon as characters to distinguish between species. Classification and the delimitation of taxa is going to become a challenge for the people working on *Eriocaulon* taxonomy because of such dubious species.

We have made efforts to resolve several species complexes with evidence from molecular and morphological data. However, considering the high intraspecific diversity and low interspecific variation within the genus versus their large number throughout the globe, we believe many more such complexes are waiting to be resolved. Recently described species have considered rootstock and stem characters to delimit species. These characters were thought to have little taxonomic value in earlier studies.

Future studies should focus on resolving the species complexes, incorporating species missing in the present study and reconstructing a high-resolution phylogenetic tree for a better understanding of the diversification of *Eriocaulon* in India. It would also be interesting to see whether the grouping of Indian species is geographically structured as reported in earlier Eriocaulaceae studies.

Acknowledgements

Authors are thankful to the Director, Agharkar Research Institute for facilities and encouragement. AMD is thankful to Council for Scientific and Industrial Research (CSIR), India for 'Senior Research Fellowship' support. SYK is grateful to the KRIBB Initiative Program of the Republic of Korea for partial funding support. Thanks are also due to the Biodiversity Boards of Maharashtra and Goa for permitting access to the biological resources for this work. Our heartfelt thanks go to all those who helped in collecting the specimens during the study. We also acknowledge the support received from the Director, Botanical Survey of India, Kolkata and curators of all the consulted herbaria for providing access to the herbarium specimens. Thanks to the anonymous reviewers, editors, Dr. Kanchi N. Gandhi, Harvard University and Prof. M.M. Sardesai, Savitribai Phule Pune University, their insightful comments helped improve the manuscript. KMP is thankful to the Director, CSIR-NBRI for facilities and encouragements.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed here: https://doi.org/doi/10.1080/14772000.2021.1914764.

Funding

This research was supported by Agharkar Research Institute (Grant No. BOT-22).

ORCID

Ashwini M. Darshetkar http://orcid.org/0000-0002-1839-8091
Mandar N. Datar http://orcid.org/0000-0002-4437-7796
K.M. Prabhukumar http://orcid.org/0000-0003-3341-2717
Soo-Yong Kim http://orcid.org/0000-0002-4608-644X
Shubhada Tamhankar http://orcid.org/0000-0002-2492-6380
Ritesh Kumar Choudhary http://orcid.org/0000-0001-6250-4624

References

Andrade, M. J. G., Giulietti, A. M., Rapini, A., de Queiroz, L. P., Conceição, A. D. S., de Almeida, P. R. M., & van den Berg, C. (2010). A comprehensive phylogenetic analysis of Eriocaulaceae: Evidence from nuclear (ITS) and plastid (psbA – trnH and trnL – F) DNA sequences. *TAXON*, *59*(2), 379–388. https://doi.org/10.1002/tax.592004

Ansari, R., & Balakrishnan, N. P. (1994;2009). *Family Eriocaulaceae in India*. Bishen Singh Mahendra Pal Singh.

Anto, P. V., & Reshma, A. (2017). *Eriocaulon pradeepii*, a new species of Eriocaulaceae from South India. *Taiwania*, *62*, 371–374. https://doi.org/10.6165/tai.2017.62.371

Biju, P., Josekutty, E. J., & Augustine, J. (2018). *Eriocaulon albotetrandra*, a remarkable new species of Eriocaulaceae from the ponds in the lateritic plateau of Western Ghats, India. *Webbia*, *73*(1), 1–4. https://doi.org/10.1080/00837792.2017.1404300

Biju, P., Subrahmanya Prasad, K., Ajith Kumar, P., Augustine, J., Raveendran, K., & Ansari, R. (2012). *Eriocaulon cheemenianum* (Eriocaulaceae), a new species from Kerala, India. *International Journal of Plant, Animal and Environmental Sciences*, *2*, 176–179.

Chandore, A. N., Borude, D. B., Natekar, P. D., Madhav, N. A., & Yadav, S. R. (2019). *Eriocaulon rayatianum*, a new species of Eriocaulaceae from the Konkan region of Maharashtra, India. *Phytotaxa*, *416*(1), 73–78. https://doi.org/10.11646/phytotaxa.416.1.9

Corriveau, J. L., & Coleman, A. W. (1988). Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. *American Journal of Botany*, *75*(10), 1443–1458. https://doi.org/10.1002/j.1537-2197.1988.tb11219.x

Darshetkar, A. M. (2021). *Molecular Phylogeny of the genus Eriocaulon L. from Western Ghats of India* [Doctoral dissertation]. Savitribai Phule Pune University, Pune, India.

Darshetkar, A. M., Datar, M. N., Rao, G. R., Tamhankar, S., Prabhukumar, K. M., & Choudhary, R. K. (2019). *Eriocaulon karaavalense* (Eriocaulaceae), a new species from India based on morphological and molecular evidence. *Annales Botanici Fennici*, *56*(4-6), 305–316. https://doi.org/10.5735/085.056.0417

Darshetkar, A. M., Datar, M. N., Tamhankar, S., & Choudhary, R. K. (2017). *Eriocaulon parvicephalum* (Eriocaulaceae), a new species from Western Ghats, India. *Phytotaxa*, *303*(3), 233–242. https://doi.org/10.11646/phytotaxa.303.3.3

Davies, R. J. P., Craigie, A. I., Mackay, D. A., Whalen, M. A., Cheong, J. P. E., & Leach, G. J. (2007). Resolution of the taxonomy of *Eriocaulon* (Eriocaulaceae) taxa endemic to Australian mound springs, using morphometrics and AFLP markers. *Australian Systematic Botany*, *20*(5), 428–447. https://doi.org/10.1071/SB07019

Doyle, J. J., & Doyle, J. L. (1987). A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochemical Bulletin*, *19*, 11–15.

Diaz Peña, C. A. (2016). *Phylogeny and biogeography of Paepalanthus subg. Platycaulon (Poales: Eriocaulaceae) in the high-Andean páramos of South America: A story of long-distance migration and rapid diversification* [Unpublished doctoral dissertation]. University of Texas.

Echternacht, L., Sano, P. T., Bonillo, C., Cruaud, C., Couloux, A., & Dubuisson, J. Y. (2014). Phylogeny and taxonomy of Syngonanthus and Comanthera (Eriocaulaceae): Evidence from expanded sampling. *Taxon*, *63*(1), 47–63. https://doi.org/10.12705/631.36

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. https://doi.org/10.1093/nar/gkh340

Francis, D., Mohan, V., Venugopal, D. K., & Nampy, S. (2020). A New Species of *Eriocaulon* (Eriocaulaceae) from the Southern Western Ghats of Kerala, India. *Edinburgh Journal of Botany*, *77*(2), 1–10. https://doi.org/10.1017/S0960428620000013

Fuertes, A. J., Rosselló, J. A., & Nieto Feliner, G. (1999). Nuclear ribosomal DNA (nrDNA) concerted evolution in natural and artificial hybrids of *Armeria* (Plumbaginaceae). *Molecular Ecology*, *8*(8), 1341–1346. https://doi.org/10.1046/j.1365-294X.1999.00690.x

Fyson, P. F. (1919–1923). The Indian species of *Eriocaulon*. *Journal of Indian Botany*, *1*, 51–53. 2, pp 133–150, 192–207, 259–266, 307–320, 3, pp. 12–18, 91–115.

Giulietti, A. M., Andrade, M. J. G., Scatena, V. L., Trovó, M., Coan, A. I., Sano, P. T., Santos, F. A. R., Borges, R. L. B., & van den Berg, C. (2012). Molecular phylogeny, morphology and their implications for the taxonomy of Eriocaulaceae. *Rodriguésia*, *63*(1), 001–019. https://doi.org/10.1590/S2175-78602012000100001

Giulietti, A. M., De Menezes, N. L., Pirani, J. R., Meguro, M., & Wanderley, MDGL. (1987). Flora da Serra do Cipó, Minas Gerais: caracterização e lista das espécies. *Boletim de Botânica*, *9*(0), 1–151. https://doi.org/10.11606/issn.2316-9052.v9i0p1-151

Govaerts, R. (2001). World Checklist of Seed Plants Database in ACCESS E-F: 1-50919.Retrieved from http://apps.kew.org/wcps/

Govaerts, R. (2020). World checklist of Eriocaulaceae. Retrieved from https://wcsp.science.kew.org/reportbuilder.do?method=Show%20Genera

Hall, T. A. (1999). BioEdit: a user–friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Hooker, J. D. (1893). *The flora of British India*. (Vol. 6). L. Reeve & Co. Ltd.

Lee, J., Joo-Hwan, K., Myong, L. S., Sang-Hong, P., Ali, M. A., Jinki, K., Sc, M., Changyoung, L., Sc, M., & Sc, B. (2009). *Seeds of wild plants of Korea*. Creseed Company.

Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F., & Donoghue, M. J. (1999). *Plant systematics: A phylogenetic approach*. Sinauer Associates. Inc.

Khanna, K. K., & Kumar, A. (2000). Taxonomic status of *Eriocaulon cinereum* R.Br. and *Eriocaulon redactum* Ruhland. *Journal of Economic and Taxonomic Botany*, *24*, 235–240.

Khanna, K. K., and., & Kumar, A. (2019). Three New Species of *Eriocaulon* L. (Eriocaulaceae) from India. *Biological Forum–an International Journal*, *11*, 21–26.

Körnicke, F. (1863). Eriocaulaceae. In Martius C.F.P. & Eichler A.W. (Eds.), *Flora Brasiliensis.* (Vol. 3, pp. 273–508). Monachii, Typographia Regia.

Kumar, R. B., Anitha, K., Watve, A., Mani, S., Rehel, S., & Arisdason, W. (2011). The status and distribution of aquatic plants of the Western Ghats. *S. Molur, KG Smith, BA Daniel, and WRT Darwall [compilers], The status and distribution of freshwater biodiversity in the Western Ghats, India*, pp.49–62.

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2017). PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, *34*(3), 772–773. https://doi.org/10.1093/molbev/msw260

Larridon, I., Tanaka, N., Liang, Y., Phillips, S. M., Barfod, A. S., Cho, S.-H., Gale, S. W., Jobson, R. W., Kim, Y.-D., Li, J., Muasya, A. M., Parnell, J. A. N., Prajaksood, A., Shutoh, K., Souladeth, P., Tagane, S., Tanaka, N., Yano, O., Mesterházy, A., Newman, M. F., & Ito, Y. (2019). First molecular phylogenetic insights into the evolution of *Eriocaulon* (Eriocaulaceae, Poales). *Journal of Plant Research*, *132*(5), 589–600. https://doi.org/10.1007/s10265-019-01129-3

Leach, G. J. (2000). Notes and new species of *Eriocaulon* (Eriocaulaceae) from Australia. *Australian Systematic Botany*, *13*(5), 755–772. https://doi.org/10.1071/SB00001

Leach, G. J. (2017). A revision of Australian *Eriocaulon* (Eriocaulaceae). *Telopea*, *20*, 205–259. https://doi.org/10.7751/telopea11531

Maddison, W. P., Maddison, D. R. (2017). Mesquite: Modular system for evolutionary analysis Version 3.31. Available at http://mesquiteproject.org.

Manudev, K. M., Robi, A. J., and., & Nampy, S. (2017). *Eriocaulon idukkianum*, a new tuberous species of Eriocaulaceae from southern Western Ghats, India. *Phytotaxa*, *324*(3), 288–292. https://doi.org/10.11646/phytotaxa.324.3.5

Moldenke, H. N. (1957). *Wurdackia* gen. nov. In B. Maguire & J.J. Wurdack (Eds.), *Botany of the chimantá. I. Gran Sabana, Venezuela*. *Memoirs of the New York Botanical Garden*, (Vol. 9 pp. 278–283). The Garden.

Naik, V. N. (1974). Some interesting species of *Eriocaulon* Linn. from India. *Journal of Indian Botanical Society*, *52*, 108–113.

Nair, R. V. (1987). Taxonomic significance of seed coat morphology in Eriocaulon Linn. (Eriocaulaceae). *Seed Science and Technology*, *15*, 297–310.

Nampy, S., Manudev, K. M., & Pradeep, A. K. (2011). Two new species of *Eriocaulon* (Eriocaulaceae) from India. *Edinburgh Journal of Botany*, *68*(2), 257–263. https://doi.org/10.1017/S0960428611000138

Naveen Kumar, V. V., Sunil, C. N., Remya Krishnan, C. R., Sanilkumar, M. G., & Baiju, E. C. (2017). A new species of *Eriocaulon* (Eriocaulaceae) from Southern Western Ghats, Kerala, India. *Webbia*, *72*(2), 155–159. https://doi.org/10.1080/00837792.2017.1369690

Okuyama, Y., Fujii, N., Wakabayashi, M., Kawakita, A., Ito, M., Watanabe, M., Murakami, N., & Kato, M. (2005). Nonuniform concerted evolution and chloroplast capture: heterogeneity of observed introgression patterns in three molecular data partition phylogenies of Asian Mitella (Saxifragaceae). *Molecular Biology and Evolution*, *22*(2), 285–296. https://doi.org/10.1093/molbev/msi016

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, *401*(6756), 877–884. https://doi.org/10.1038/44766

Pagel, M., Meade, A., & Barker, D. (2004). Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology*, *53*(5), 673–684. https://doi.org/10.1080/10635150490522232

Paithane, V. A., Bhutkar, A. S., Kashetti, R. P., & Patil, S. B. (2017). A new species of *Eriocaulon* (Eriocaulaceae) from lateritic plateaus of Sindhudurd, Maharashtra, India. *International Journal of Advanced Research*, *5*, 858–862.

Phillips, S. (1997). *Flora of Tropical East Africa-Eriocaulaceae*. CRC Press.

Phillips, S. M. (1994). Notes on some *Eriocaulon* species from Ceylon. *Kew Bulletin*, *49*(2), 287–303. https://doi.org/10.2307/4110265

Prajaksood, A., Chantaranothai, P. & Parnell, J. A. N. (2017). Eriocaulaceae. In: T. Santisuk & H. Balslev (eds), *Flora of Thailand* (Vol. 13, pp. 434–511). Prachachon Co. Ltd.

Punekar, S. A., Malpure, N. V. and Lakshminarasimhan, P. (2003). Five new species of *Eriocaulon* L. (Eriocaulaceae) from Western Ghats, India. *Rheedea*, *13*, 19–27.

Punekar, S. A., Malpure, N. V., & Lakshminarasimhan, P. (2004). Three new species of *Eriocaulon* (Eriocaulaceae) from peninsular India. *Sida*, *21*(2), 625–635.

Rambaut, A. (2017). *FigTree–version 1.4. 3, a graphical viewer of phylogenetic trees*. Computer program distributed by the author, Retrieved from: http://tree.bio.ed.ac.uk/software/figtree.

Rambaut, A., Drummond, A. J., Suchard, M. (2014). Tracer v1. 6 Retrieved from: http://beast.bio.ed.ac.uk.Tracer.

Rashmi, K., & Krishnakumar, G. (2014). *Eriocaulon gopalakrishnanum* sp. nov.(Eriocaulaceae) from the Western Ghats, India. *Nordic Journal of Botany*, *32*(2), 146–149. https://doi.org/10.1111/j.1756-1051.2012.00030.x

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029

Rosa, M. M., & Scatena, V. L. (2003). Floral anatomy of Eriocaulon elichrysoides and Syngonanthus caulescens (Eriocaulaceae). *Flora–Morphology, Distribution, Functional Ecology of Plants*, *198*(3), 188–199. https://doi.org/10.1078/0367-2530-00090

Rosa, M. M., & Scatena, V. L. (2007). Floral anatomy of Paepalanthoideae (Eriocaulaceae, Poales) and their nectariferous structures. *Annals of Botany*, *99*(1), 131–139. https://doi.org/10.1093/aob/mcl231

Roskov Y., Ower G., Orrell T., Nicolson D., Bailly N., Kirk P.M., Bourgoin T., DeWalt R.E., Decock W., Nieukerken E. van, Zarucchi J., Penev L., eds. (2019). Species 2000 &

ITIS Catalogue of Life, 2019 Annual Checklist. Retrieved from www.catalogueoflife.org/annual-checklist/2019

Ruhland, W. (1903). Eriocaulaceae. In: Engler A, ed. *Das Pflanzenreich*. (Vol. 4. pp. 1–294). Wilhelm Engelmann.

Sambrook, J., & Russell, D. W. (2001). *Molecular cloning: a laboratory manual*. Cold Spring Harb Lab Press.

Schluter, D., Price, T., Mooers, A. Ø., & Ludwig, D. (1997). Likelihood of ancestor states in adaptive radiation. *Evolution; International Journal of Organic Evolution*, *51*(6), 1699–1711. https://doi.org/10.1111/j.1558-5646.1997.tb05095.x

Shimpale, V. B., & Yadav, S. R. (2010). *Eriocaulon belgaumensis*: a new species of Eriocaulaceae from the Western Ghats of India. *Kew Bulletin*, *65*(2), 337–339. https://doi.org/10.1007/s12225-010-9204-5

Shimpale, V. B., Bhagat, R. B., Deshmukh, R. B., & Yadav, S. R. (2009). A new species of Eriocaulon (Eriocaulaceae) from Maharashtra, India. *Rheedea*, *19*(1), 47–49.

Stützel, T. (1998). Eriocaulaceae. In K. Kubitzki (Ed.), *Flowering Plants·Monocotyledons.* (pp. 197–207)Springer.

Sun, Y., Skinner, D. Z., Liang, G. H., & Hulbert, S. H. (1994). Phylogenetic analysis of Sorghum and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *89*(1), 26–32. https://doi.org/10.1007/BF00226978

Sunil, C. N., & Kumar, V. N. (2015). A new species of *Eriocaulon* (Eriocaulaceae) from Western Ghats, India. *Webbia*, *70*(2), 211–215. https://doi.org/10.1080/00837792.2015.1050175

Sunil, C. N., Narayanan, M. R., Nandakumar, M. K., Sujana, K. A., Joseph, J. P., & Kumar, N. A. (2013). *Eriocaulon kannurense* (Eriocaulaceae), a new species from Kerala, India. *International Journal of Plant, Animal and Environmental Sciences*, *3*, 116–120.

Sunil, C. N., Narayanan, R. M. K., Kumar, V. N., Nandakumar, M. K., & Balakrishnan, V. (2017). *Eriocaulon govindiana* sp. nov. (Eriocaulaceae), from southern Western Ghats, Kerala, India. *Taiwania*, *62*, 387–391. https://doi.org/10.6165/tai.2017.62.387

Sunil, C. N., Ratheesh Narayanan, M. K., Sivadasan, M., Alfarhan, A. H., & Abdul Jaleel, V. (2015). *Eriocaulon vandaanamense* sp. nov. (Eriocaulaceae) from Kerala, India. *Nordic Journal of Botany*, *33*(2), 155–158. https://doi.org/10.1111/njb.00658

Swapna, M. M., Rajesh, K. P., Manju, C. N., & Prakashkumar, R. (2012). *Eriocaulon madayiparense* (Eriocaulaceae)–A new species from the foot hills of the Western Ghats of India. *PhytoKeys*, *10*(0), 19. https://doi.org/10.3897/phytokeys.10.2297

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*(5), 1105–1109. https://doi.org/10.1007/BF00037152

Trovó, M., De Andrade, M. J. G., Sano, P. T., Ribeiro, P. L., & Van den Berg, C. (2013). Molecular phylogenetics and biogeography of Neotropical Paepalanthoideae with emphasis on Brazilian *Paepalanthus* (Eriocaulaceae). *Botanical Journal of the Linnean Society*, *171*(1), 225–243. https://doi.org/10.1111/j.1095-8339.2012.01310.x

Unwin, M. M. (2004). *Molecular systematics of the Eriocaulaceae Martinov.* [Doctoral dissertation, Miami University]. https://etd.ohiolink.edu/pg_10?0::NO:10:P10_ACCESSION_NUM:miami1082582823

Uyeda, J. C., Zenil-Ferguson, R., & Pennell, M. W. (2018). Rethinking phylogenetic comparative methods. *Systematic Biology*, *67*(6), 1091–1109. https://doi.org/10.1093/sysbio/syy031

Vaidya, G., Lohman, D. J., & Meier, R. (2011). SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*, *27*(2), 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Vivek, C. P., Swapna, M. M., & Suresh, K. K. (2010). *Eriocaulon wayanadense* (Eriocaulaceae), a new species from Kerala, India. *Rheedea*, *20*, 25–27.

Watanabe, M. T. C., Hensold, N., & Sano, P. T. (2015). *Syngonanthus androgynus*, a striking new species from South America, its phylogenetic placement and implications for evolution of bisexuality in Eriocaulaceae. *PloS One*, *10*(11), e0141187. https://doi.org/10.1371/journal.pone.0141187

Wendel, J. F., Schnabel, A., & Seelanan, T. (1995). Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (Gossypium). *Proceedings of the National Academy of Sciences*, *92*(1), 280–284. https://doi.org/10.1073/pnas.92.1.280

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: a Guide to Methods and Applications*, *18*, 315–322. https://doi.org/10.1016/b978–0–12–372180–8.50042–1

Yao, J. L., Cohen, D., & Rowland, R. E. (1994). Plastid DNA inheritance and plastome-genome incompatibility in interspecific hybrids of Zantedeschia (Araceae). *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *88*(2), 255–260). https://doi.org/10.1007/BF00225906

Zhang, Q., & Liu, Y. (2003). Examination of the cytoplasmic DNA in male reproductive cells to determine the potential for cytoplasmic inheritance in 295 angiosperm species. *Plant and Cell Physiology*, *44*, 941–951. https://doi.org/10.1093/pcp/pcg121

Zhang, Z. (1999). *Monographie der Gattung Eriocaulon in Ostasien.* [Doctoral Dissertation]. Berlin and Stuttgart: J. Cramer.

Associate Editor: Mark Carine